ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

FISCAL YEAR 1971

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

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July 1, 1970 through June 30, 1971

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NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

OFFICE OF DIRECTOR

At the end of Fiscal Year 1971, the National Institute of Child Health and Human Development had been in existence eight and a half years.

Though definitive answers to some of the major problems with which the Institute has been concerned are still a few years away, it is clear that the potential impact of NICHD supported research findings upon the scientific community and the lives of large portions of our population is great.

Years of careful work in the field of nutrition is beginning to bear fruit. Undoubtedly within the next few years we will have clear indications of how the intellectual development of a child can be affected by his mother's malnutrition - or optimal nutrition - during pregnancy.

How to cope with the deficiencies exhibited by children born into disadvantaged homes and communities is being productively studied in a number of institutions. These efforts will eventually improve the quality of life for a great many individuals, and a great many families.

Research efforts within the Institute's laboratories, and through grants and contracts supported by the Institute, show promise of producing results which will enable our population to undertake more wisely the governance of their own fertility.

Intensive investigations of all aspects of prenatal life will result in the birth of fewer babies handicapped by faulty early development.

At the other end of the life span, careful work designed to lead to an understanding of the process of aging on the cellular level will open research approaches that may eventually lead to more vital living for millions of older citizens.

In the previous year, Fiscal Year 1970, the Institute experienced a 2% increase in the dollar amount available for funding all types of research and training grants and contracts, and a 12% decrease in the number of projects funded. This year Institute funds available for funding all types of research and training grants and contracts and research contracts amounted to $76.8, an increase of 26% ($16.0 million) from the 1970 portfolio, and a decrease of less than 1% in numbers of projects funded.

Even with these increased funds, over 500 approved applications, representing an estimated dollar value of over $22 million, could not be funded.

Despite the very slight increase in total number of projects funded, fewer research grants, training grants and fellowships were awarded in Fiscal 1971 than in 1970. Major increases were experienced in research contract funding, with an increase of almost 44% in the number of projects funded, and 115% in

amount of dollars. The Center for Population Research accounted for 175 research contracts and reimbursable agreements in the amount of $13.1, and 35 research contracts in the amount of $3.5 million for the remainder of the Institute's programs. The table which follows points up this situation.

Type of Award	No. of Projects	+ or - over 1970	Amount of Dollars (millions)	+ or - over 1970
Research Grants	745	-54	$42.3	+$4.4
Training Grants	118	-13	10.0	+ 0.7
Fellowships	75	-11	0.8	- 0.1
Research Career and RCDA	116	+ 3	3.0	+ 0.1
Research Contracts	210	+64	16.6	8.9

Office of the Associate Director for Program Planning

The Office of the Associate Director for Program Planning and Evaluation spent a major portion of its time in assessing and reviewing past NICHD activities to provide a framework for formulating long-range goals in terms of the Institute's mission and the needs of the '70s and '80s. The basic issue relates to the Institute's orientation, focus, and programmatic directions that will lead to achievements and directions relevant to the world ten and twenty years hence. Here the challenge is to provide alternative goals and objectives that remain flexible and responsive, are subject to continuing review, and yet at the same time provide a firm definition of the Institute's mission.

Specifically, Fiscal Year 1971, Congressional and interagency interest led to the preparation of several reports on aspects of the Institute's research and training programs with particular emphasis being given on how activities pertaining to early child development interface with other Federal efforts in this area.

Other issues to which the Office addressed itself included the following: factors in the man-made environment with a serious impact on child development; the need to expand research efforts into the causes and prevention of the Sudden Infant Death Syndrome; research concerning the effects of medications administered during pregnancy and childbirth on the developing infants' behavioral and physiological functioning.

The facilities and resources activity of the OPPE has included the function of coordinator of planning for Building 33, the NICHD Research Facility to be built on the NIH campus. This building, now in the final stages of design, will contain approximately 90,000 net square feet. It also includes participation in the development of plans for an intramural clinical research unit to be activated in the Clinical Center, NIH, via an addition of 15,492 net square feet to be made available through construction of an 8th, 9th, and 10th floor on Wing G. Expected date for occupancy is June 1974.

The Acting Associate Director, Dr. Samuel Greenhouse, is representing the

Institute at the NIH Advisory Committee for Program Planning and Evaluation. Dr. Greenhouse was also asked to be Chairman of the evaluation Sub-Committee of the parent Advisory Committee and has served in this capacity since becoming Acting Associate Director.

Office of Public Information

At the conclusion of Fiscal Year 1971, the Public Information Office was realigning office responsibilities and emphases to maximize the effectiveness of the present staff. Based on three years experience at the Gerontology Research Center in Baltimore and two years' experience with the Center for Population Research, it was evident that organizing information activities around unit or program activities made for effective staff use. Such an organization, it is felt, is particularly useful in NICHD, which has a wide and highly disparate group of programs.

Among the highlights of public information activities last year included the issuance of a pamphlet, Population Research, covering all of the Institute's population activities. Its excellence is such that it was awarded a Blue Pencil Award in the Federal Editor's Association's annual publications contest.

Following a rapid growth in the number of press and public inquiries handled by the Public Information Office in the last few years, fiscal year 1970-71 saw a levelling off of these inquiries. Approximately 250 press inquiries from about 90 media outlets were handled, and approximately 8,000 public inquiries. About 85,000 publications were distributed, over 100 features and press releases prepared, 19 radio and television appearances arranged for, 111 Congressional inquiries responded to.

Of prime importance to future public information activities will be the careful development of sound information activities related to the Institute's intramural programs at Bethesda.

Epidemiology and Biometry Branch

Fiscal 1971 was the first year the Epidemiology and Biometry Branch was organized into two sections: a Biostatistics Section and an Epidemiology Section. This organization reflected the increasing functions and activities in each of these two major areas of responsibility, with the Biostatistics Section assuming the mathematical statistical activities of the Branch, such as providing consultation in the design and analysis of experiments, and in carrying on research in statistical methods.

The Branch has been involved in two significant developments concerning registries. One concerns the technique of amniocentesis which is being used more and more frequently to monitor the fetus of a high risk mother. The Branch was represented at a series of meetings initiated by the Scientific Director to plan the establishment of a central registry to assess the risk of the technique, and has assumed the responsibility for maintaining this registry and for the analysis of the data. The Branch has also given very serious consideration to undertaking a similar role with regard to a birth defects registry. A group of such registries, representing different regions

of the United States, could serve as a surveillance device for detecting teratogenic environmental agents, and constitute a useful data base for specific research studies.

Staff of the Epidemiology Section served as project officers on three research contracts, reviewed all literature concerned with the epidemiology of respiratory distress syndrome of the newborn, and also with the epidemiology of mental retardation.

Staff of the Biostatistics Section, besides being involved in activities related to the amniocentesis registry and the birth defect reporting system were project officers on five research contracts. Research activities in statistical methods have included the following: the problem of transformations in the analysis of variance; time series analysis and the problem of robust spectrum estimation; the circumstances in which matching is appropriate in case-control studies; the ability of three epidemiologic instruments to detect increased risk of disease resulting from a suspected agent.

Center for Population Research

Fiscal Year 1971 was the second full year of operation of the Center for Population Research. The Center's grant and contract programs supported research and training in a wide range of areas in the biomedical and social sciences. Special emphasis was given during the year to three high priority topics: (1) the development of new contraceptive methods, (2) the evaluation of fertility regulating methods currently in use, and (3) the social science aspects of population research. The number of professionals on the Center's staff increased from fifteen to twenty-two, and estimated budget increases for FY 1971 over FY 1970 were 48%.

The Center's contraceptive development program is one of the most important and visible population research activities currently underway, since the need for an array of new methods of fertility regulation is clearly demonstrated. Initial efforts in contraceptive development consisted of the support of basic research in a limited number of areas in reproductive biology which showed special promise for the development of new contraceptive methods. This program is being continued, and, in addition, a directed program of more applied research has been launched. Contracts have been negotiated for studies on improved techniques of sterilization and improved drug delivery systems, and the initiation of a drug development program involving synthesis, testing, and ultimately clinical trials of promising new chemical compounds. Staff members have explored potential problems relating to patent policies and government-industry relationships, looking forward to making satisfactory contractual arrangements with industrial concerns as well as private agencies.

The evaluation of the medical effects of contraceptives presently in use represents a continuation of one of the earliest activities of the CPR. A new Fertility Regulating Methods Evaluation Branch has been established and high priority is being given to initiation of biochemical and pharmacological investigations of the oral contraceptives. This program includes studies of metabolism and dose-response relationships as well as biochemical investi-

gations of effects on systems controlling blood pressure and on endocrine function, vitamin and mineral metabolism, and interactions with other drugs.

The Behavioral Sciences Branch has continued and expanded its programs of contract research in the social sciences. The primary aim of the social science program is the expansion of knowledge of (1) the relationships between population size, growth, composition and distribution and the general welfare or quality of life measured from a variety of points of view; and (2) the social, psychological, economic and other factors which influence birth rates (thus affecting rates of population growth) and migration patterns (which affect population distribution).

This past year saw the initiation of a new program of grants for Population Research Centers which was announced in July 1970. After extensive staff work to inform prospective applicants of the guidelines for these new grants, six applications were reviewed at the March 1971 meeting of the NACHD Council and four more were reviewed in June.

Center grants, which provide funds for core services, personnel and for development of new areas of population research in academic and non-profit institutions with established expertise in the population field, will make an important contribution to long-term institutional support for population research.

As of January 31, 1971, Population and Reproduction Grants Branch funding of $11.7 million supported 193 research grants, 29 training grants, 37 fellowships and 18 research career development awards. Approximately 83 percent of these funds support research in the area of reproductive biology and contraceptive development, 9 percent in animal behavior and ecology, and 8 percent in social aspects of population research.

Although funds available for research grants increased in 1971, funds for training grants in the population field shared in the critical scarcity of NIH training funds, and the Center was able to fund only one new and two renewal applications of 19 competing applications approved by the reviewing groups. This situation, if continued, will necessarily restrict the needed expansion of research activities in both the biomedical and the social sciences.

The Center has long had an interest in developing collaborative research projects with foreign scientists under the PL-480 programs, and this activity has progressed significantly. The Center now supports four active projects. Five others have been approved by NIH and now await formal approval by the foreign government. This program may be considerably expanded next year, as indicated by the fact that 39 projects are now in various stages of preliminary development.

The Center's information and liaison activities with other agencies in the population field, which have been conducted by the Program Liaison Branch, will be transferred to the Office of the Director of the Center when the present Chief of the Branch retires on June 30, 1971. Other Center activities included the support of several conferences: on oogenesis; on the biology of the cervix; on research approaches to sex education, contraception, family

planning and morality. A joint Conference was held with the Fogarty International Center on the regulation of mammalian reproduction.

Since 1967 the Center for Population Research and its predecessor units in the NICHD have received invaluable advice and guidance in the development of new programs and evaluation of ongoing ones from the Population Research Advisory Committee, a group of some 17 experts in the biomedical and social science aspects of the population field. In 1971, the Office of the Secretary decided to establish a Secretary's Committee on Population Affairs, with task forces for research and for family planning activities to provide the advice required by the CPR and the National Center for Family Planning Services. The Population Research Advisory Committee was therefore disbanded after a final meeting in January 1971.

Office of Associate Director for Program Services

An increase in the contract management activities and the realignment of the mission of the Program Statistics Branch and the Scientific Information Centers Branch were major factors in the Institute's Office of Program Services during the year.

A total of 210 contracts which have a value of about $16.6 million have been processed, more than double the $7.7 million processed the previous year. At the same time, while there was a small decline in the number of research grants processed (54), there was an increase of $4.4 million over the 1970 total, for a 1971 total of $46.3 million. Training grants remained about stationery in 1970, with 118 awards made for a total value of about $10.0 million. The total portfolio of research and research training grants, research fellowships, research career and research career development awards and research contracts was about $76.8 million in Fiscal Year 1971. This represents an increase of $14.0 million, or 22% over Fiscal Year 1970.

The termination of publication of the two experimental abstract journals provided the Institute with the unusual opportunity of evaluating the priorities in Program Statistics and the Scientific Information Centers Branch. This resulted in a reorganization and the title of the Scientific Information Centers Branch was changed to Scientific Information and Publications Branch with greater emphasis on the management of scientific publication reference services.

The Reference Service Section acquires, catalogues and maintains library material for use by NICHD staff. Last year the Section handled the purchase of over 1,300 books and filled almost 1,400 interlibrary loan requests. The Scientific Publications Section provides central service for the editing, composing and designing of the Institute's scientific publications.

The Technical Publications Section of the Scientific Information Centers Branch issued the proceedings of a conference Issues in Human Development, edited by Dr. Victor Vaughan, and also published Sudden Infant Death Syndrome, a summarization of a scientific conference proceeding previously issued by the University of Washington Press. It also published the third annual survey of population research and research related activities entitled Population

Research: 1969.

The Program Statistics Branch became the Program Statistics and Analysis Branch with added emphasis on research analyses and information systems. The Research Analysis Section of this Branch is responsible for the development and printing of grant and contract summaries including research progress and the analysis of research accomplishments of NICHD grantees. The Information Systems Section is responsible for the development of systems for the storage and retrieval of data relevant to the Institute's programs and for the coordination of computer activities within NICHD and of NIH and external facilities as required.

The Scientific Conference Branch in addition to assisting in the planning and arranging of 21 conferences under the direct sponsorship of NICHD also assisted in the management of eight NICHD Advisory Committees which includes the National Advisory Child Health and Human Development Council. Of the approximately 525 scientists who participated in the 21 meetings, almost half of them were first time participants in Institute conferences.

Office of the Associate Director for Intramural Research Programs

The programs of intramural research within the Institute have, during the past year, shown progress in a number of areas and contributed significantly to the acquisition of new knowledge in problems of development and aging. Reorganization of the Gerontology Research Center has placed the Branch Chiefs in a position to exercise direct responsibility for the program content in a way similar to that enjoyed by Branch Chiefs on the Bethesda campus. The reproduction research program has expanded markedly by the transfer of the Endocrinology Branch of NCI to NICHD. Dr. Mortimer Lipsett was made Associate Scientific Director for Reproduction while retaining his position of Chief of the Reproduction Research Branch.

In general, programs have shown considerable consolidation and branches, newly organized during the previous fiscal year, have begun to function effectively. A Pregnancy Research Branch was established to begin operations in Fiscal Year 1972. A Clinical Director, Dr. John Robbins, joined the staff. He has begun intense research activity in the pathogenesis of infections in human infants. Within the next fiscal year his program will be incorporated into a Developmental Immunology Branch.

Total budgeted positions in intramural research has increased from 224 to 244. In addition, a total of 69 scientists are working in the Institute laboratories as guest workers, guest scientists, visiting fellows, visiting scientists, and visiting associates.

While the dispersed nature of the activities of the Institute have presented many handicaps, it has been possible to slowly weld the branches into an organized unit with interplay and interaction among the component parts.

Brief descriptions follow of the more important activities and accomplishments in each laboratory.

Office of Clinical Director

The research activities of the Office of the Clinical Director are concerned with two scientific problems. The first is the disease caused by Hemophilus Influenzae type b in humans. Several research programs have been designed to study the mechanisms of the pathogenesis, host susceptibility (acquired and constitutive), and the prevention of this disease.

The second research problem is biologic role of serum alpha-1 antitrypsin inhibitor. This serum protein is present in high concentration in normal humans. The genetic control of its structure, its function in vivo and its precise relation to the development of the pulmonary disease in young adults with genetically directed deficiency of this serum protein are the problems under consideration.

During the year, microorganism possessing cross reacting antigens with the Hemophilus Influenza have been identified. These antigens appear to be products of microbial metabolism rather than structural elements as in the case of the capsular polysaccharide of H. Influenza. It has been demonstrated in rat models that an antigen derived from E. Coli can protect against lethal infections with H. Influenza. These experiments form the basis for proposing that much of the so-called "natural immunity" to known pathogenic organisms in man and animals is derived not from infection but, rather, as a result of antibody production in response to antigens produced by unrelated organisms.

Developmental Biology Branch

The Developmental Biology Branch continues under its present structure with an Acting Branch Chief. The programs of the laboratory are concerned largely with early molecular events associated with fertilization and biochemical changes and control mechanisms for differentiation. The principal models have been the chick embryo and the sea urchin egg.

It has been observed that the lens from the eye of a six day old chick, when excised and grown in tissue culture, dramatically reduces the complexity of the protein profiles that are synthesized; within three days only a single species, that with a molecular weight of 45,000 is produced. The events in tissue culture closely mimic those found in normal embryonic development. This particular model can be exploited for the understanding of the differentiation which occurs when a single protein is made. Eventually, this single protein becomes lens crystalline.

Reproduction Research Branch

The mission of the Reproduction Research Branch is the development of scientific data and theory basic to reproductive biology. Research efforts therefore extend from the chemistry of the protein and steroid hormones to physiologic and clinical studies in man.

During the past year a fundamental question about gonadotropic hormones has been resolved by use of tissue culture of human trophoblast cells. It has

been shown that gonadotropin heterogeneity is due, in large part, to the synthesis of gonadotropins with different sialic acid content.

The pace of clinical investigation has continued and this year male and female infertility clinics were established in addition to the Endocrinology Clinic. The first evidence has been obtained that, in man, germinal epithelium activity modulates the release of FSH, a most important finding for studies of control of spermatogenesis.

Social and Behavioral Sciences Branch

The Social and Behavioral Sciences Branch has been engaged in a number of investigations in which applied and theoretical interests converge. The focus of the research program is on early environmental influences on cognitive and personality development. Theoretical issues concerning early experiences have a special relevance at the present time as programs are being developed to overcome and mitigate the effects of extreme deprivation on young children growing up in impoverished environments.

In a study of environment of six month old infants from poorer socioeconomic backgrounds, it was found that it may not be very meaningful to label environments grossly as depriving. Relatively low relationships between dimensions of the inanimate and social environments have been found. Specific dimensions of the early environment are significantly related to specific aspects of infant functioning.

Behavioral Biology Branch

Within the Behavioral Biology Branch, a pilot study of some behavioral, electrophysiological and biochemical consequences of early sensory and social deprivation has been nearly completed.

The EEG of naturally deprived rhesus monkeys show abnormal patterns, particularly in the slow wave stage of sleep indicating a diffuse cerebral dysfunction rather than abnormalities of the specific sensory or motor systems. No abnormalities in blood platelet serotonin levels have been found.

Laboratory of Biomedical Sciences

Recent work in the Laboratory of Biomedical Sciences has delineated genetically the mechanism involved in the insertion and excision of the phage λ into and from its host's genome. By removing the usual attachment site for this phage, it has also been possible to insert into many points the host genome and thus to produce a wide variety of phage, containing small portions of the host chromosome. These are vital to subsequent studies requiring a relatively purified set of genes and should prove extremely useful to the field in general.

Six placental enzymes are under study: glucose dehydrogenase; lactic acid dehydrogenase; glucose-6-phosphate dehydrogenase; 6-phospho gluconate dehydrogenase; beta glucuronidase and hexosaminidase. Methods have been devised for demonstrating isoenzyme patterns of the first three enzymes.

During growth and development, the enzymes may show developmental variations in isoenzyme pattern, in total activity, or in both.

In other sections of the Laboratory, it has been shown that rat brain uses uridine in preference to orotic acid as a precursor of hyrimidines, and an extremely sensitive assay for cyclic AMP has been developed in order to study the effects of glucose-6-phosphate and glycogen in the regulation of glycogen synthetase phosphatase.

Children's Diagnostic and Study Branch

The program content of the Children's Diagnostic and Study Branch during the past year has been related in large measure to cytogenetic studies, studies of lineage through dermatoglyphics and various proteins of plasma, and reading disability.

Among the Branch's accomplishments has been the completion of a study of perceptual functioning in a group of children identified as having reading disability. After screening to eliminate those with discernable neurological problems, it was found that only one of the 54 was a female. All subjects showed superior ability to perform visual ordering of material and information. The studies suggest that there is a hereditary component transmitted by an x-link recessive gene which confers upon males this ordering superiority but in addition confers inefficiency in reading.

Gerontology Research Center

During the year considerable progress has been made in developing new knowledge of understanding the basic mechanisms of aging. This included data derived from studies conducted at various levels: molecular, cellular, organ and whole animal.

Within the Clinical Physiology Branch, a non-invasive technique for the assessment of ventricular function by computation of systolic time intervals has been applied to 333 normal men aged 20-93. In subjects with no clinical evidence of heart disease, the total ejection time and the pre-ejection phase increase to a maximum at age 60. After age 60 both variables decline. Since these increasing values may reflect a decrement in ventricular function with age, the failure of average values to increase after age 60 may be a reflection of selective mortality, subjects with high values may have higher mortality rates in later life so that cross-sectional data may not show a further increase after age 60.

In the Laboratory of Behavioral Sciences, ongoing experiments with monkeys are assessing the relation between state of arousal, and direction of control of pulse rate. Animals are trained to speed and slow heart rate. At the same time that the animal is learning heart rate control, or performing at a high level of learning, the percentage time during which it is producing high voltage, synchronized brain waves is also being recorded. Preliminary findings suggest that voluntary cardiac slowing is associated with a relatively "relaxed" brain while voluntary cardiac speeding is associated with a more "aroused" brain.

Studies at the Laboratory of Cellular and Comparative Physiology have shown that, with increasing age, cells in a variety of animals (rotifers, crayfish, mice, rats) show an increased accumulation of lysosomes and lysosomal enzymes which break down cytoplasmic substances. Similar increases in lysosomal enzymes have been found in cells or organs undergoing hormone induced involution. Thus further evidence is accumulating to indicate the importance of lysosomes in cell loss which is associated with aging.

The Laboratory of Molecular Aging has used the healing surgical wound as a model system to examine the mechanisms of aging of soft connective tissues. Young animals, rats of 8 months of age, produce a significantly greater mass of scar tissue in response to incision than do 18 month old rats, and this is reflected in an increased breaking strength for the scar tissue of the younger animal. The tissue produced by the younger animal is more compliant (extension at break is greater) than is that produced by the older animals.

Collaborative Guest Scientist Program

During the past year the Collaborative Guest Scientist Program has been initiated. Eight guest scientist programs have been established in the Center. These programs are presently staffed by 19 scientists and 12 technicians and have underway 7 projects. Several important observations have been made by scientists working in this program.

For example, the non-intestinal effects of cholera toxin administered intravenously to dogs, mice and rats have been studied. Biochemical determinations on serum and specific tissues (liver, blood platelets) add to the growing evidence that cholera toxin alters cell function by activating adenyl cyclase activity. The effect of the toxin is delayed in onset, prolonged duration and active on a variety of cell types. This suggests that cholera toxin may prove a unique tool for the manipulation and study of adenyl cyclase-camp mediated processes. It may also be extremely useful for studying the functions of aging cells and organs.

Office of the Associate Director for Extramural Programs

Programs of research and training in Perinatal Biology and Infant Mortality, Growth and Development, Mental Retardation, and Adult Development and Aging were continued during Fiscal Year 1971. At the beginning of the year, all research and training projects in developmental biology were transferred from the Center for Population Research to the Perinatal Biology and Infant Mortality Branch.

There were two significant developments in the roles and activities of the Institute's Research and Training Committees during the year. First, the Research and Training Committees were given the responsibility for providing initial review for program projects in addition to training grants, research career development awards and fellowships. And, second, Chairmen of the Committees were invited to attend meetings of the NACHD Council, beginning with the March 1971 meeting.

Despite the fact that the number of approved applications which cannot be

funded is growing, the Institute's Extramural staff has continued to engage in the active programming of applications. In addition to continuing these efforts, the extramural scientific staff intensified its efforts to encourage certain types of research via the contract mechanism. Midway through the year a new requirement was initiated. Contract proposals must now be sent out to a number of prospective contractors even when the research is to be done in a university. The resulting delays in the reviewing process were balanced by the fact that the quality of contracts eventually approved was improved.

The fact that support of research training continued to become more and more difficult during Fiscal Year 1971 was especially hard on the NICHD because many of the areas the Institute is responsible for are relatively new on the academic scene--for example, perinatology and mental retardation. The number of investigators needed continues to increase in spite of the fact that funds for training grants, research career development awards and fellowships do not increase.

All of the Extramural Programs continued to hold conferences during the year, from many of which publications will eventually appear. These are regarded as extremely important aspects of the work of the Institute.

Highlights of the activities and accomplishments of the extramural branches are detailed in the reports which follow.

Perinatal Biology and Infant Mortality Branch

The objective of the Perinatal Biology and Infant Mortality Branch is the promotion of a coordinated program of research and training which will result in the contribution of new knowledge toward the achievement of maximum health and well-being for every pregnant woman and her progeny. Program objectives also strive to achieve a marked reduction in this country's infant mortality rate, to ameliorate infant morbidity, and to narrow the gap between the identification of new knowledge and its incorporation into the delivery of health care.

The Perinatal Biology and Infant Mortality Branch is responsible for the supervision of approximately 25 percent of the Institute's extramural holdings, totaling about 299 grants and representing over 12.4 million dollars. Approximately one quarter of the Program's grant holdings are concerned with training support.

The Perinatal Biology and Infant Mortality Program is organized into four sub-programs: Pregnancy and Maternal Health, Developmental Biology, Fetal Health and Development, and Infant Survival and Well Being.

Among the scientific accomplishments achieved by research in the Pregnancy and Maternal Health area are the findings of Dr. Flossie Cohen at the Children's Research Center of Michigan. She has recently found that some transplacental hemorrhage occurs following amniocentesis. She has found fetal cells in the maternal circulation following this procedure in 20 to 30 percent of the cases studied. Furthermore, complications following amniocentesis include an increase in maternal antibody 30 to 40 percent of

the time.

In the Developmental Biology area, Dr. Gluecksohn Waelsch at the Albert Einstein College of Medicine has found that radiation-induced albino mutations cause death of homozygotes within several hours after birth as a result of hypoglycemia. The cause of the severe decrease in blood sugar levels has been identified as the absence of glucose-1-phosphatase activity, which does not appear until just before the time of birth. Before this time, fetal blood sugar levels are controlled by the maternal organism. A considerable proportion of homozygotes displayed morphogenetic abnormalities, e.g., of the thymus and of the kidney. One further property of the radiation-induced mutations was the absence of a dosage effect in heterozygotes. This work is important for it provides valuable insight into genetic expression in the clinical situation.

Within the Fetal Health and Development area, Dr. Andre Hellegers of Georgetown University, Washington, D. C., has conducted in vivo and in vitro studies of fetal oxygenation. He has reported that by diffusion respiration, the fetus is capable of surviving for approximately three quarters of an hour in the total absence of maternal breathing. This observation led him to study the fetal conditions of oxygen transport, particularly those associated with sickle-cell anemia. Dr. Hellegers reports that in sickle-cells he has found the 2, 3 DPG content to be increased in amounts which are not incompatible with changes in oxygen dissociation curves observed in sickled whole blood.

Two interesting research findings resulting from grants in the Infant Survival and Well Being area are highlighted in the following paragraphs:

Dr. Michael Heyman, a Research Career Development Awardee at the University of California, is investigating the possible pharmacological induction of closure of the ductus arteriosus in premature infants. As a result of his experimental studies with sheep, it has been shown that the premature ductus arteriosus is less responsive to oxygen than in the mature fetal lamb. The ductus does however constrict with acetylcholine. Dr. Heyman is now investigating the use of acetylcholine injected at the aortic orifice of the ductus arteriosus in closing the patent ductus in human premature infants in cardiac failure.

Dr. Roderic Phibbs, another Research Career Development Awardee of the University of California, has continued his follow-up evaluation of infants who received intrauterine transfusions during fetal life. He has found the tendency for functional performance to improve with age. This continued improvement was unexpected and is of particular interest.

Growth and Development Branch

The Growth and Development Branch supported approximately 30% of the research grants in the NICHD during FY 1971. This percentage is a decrease from about 35% several years ago. The change is accounted for by the increase in support in other programs within NICHD and the disproportionate increase in average grant size vis-a-vis increased in overall budget. During this fiscal year

special attention has been focused on nutrition and on early child development, with these areas representing absolute increases in both number of grants and amounts of money invested.

The Branch was responsible for issuing two significant new books last year--Issues in Human Development, which explores the biomedical and environmental factors which shape the development of children and adults, and The Secretory Immunologic System. The latter, a highly technical report, brings together the observations of scientists concerned with secretory antibodies in many different organs, with emphasis on the relationship between these and the development of resistance to disease.

Within the area of nutrition, two grant-supported projects are investigating the interrelationships between socioeconomic class, environmental opportunities, malnutrition in mothers and infants, and behavioral outcomes during the first 5-7 years of life. One of these has just gotten underway. The other reports a reduction in brain size in children born to severely malnourished mothers; subsequent behavioral development is retarded but may partially respond to subsequent rehabilitation with a combined nutrition and education program. However, the nutritional rehabilitation must start very early in life. These studies are being done in a country where breast feeding of infants has been almost entirely abandoned. NICHD is supporting a project in another country where attempts to assess the nutritional impact of prolonged breast feeding are underway. For the first time this study has shown a decrease in the quality of mother's milk as a function of maternal malnutrition, with a consequent adverse effect on infant growth. The results in terms of psychological development have not yet been measured.

Research supported for several years in the learning and cognitive development area by the Growth and Development Branch has uncovered much that was not previously known about the nature of intellectual functioning, and ways to assess it. For example, it has been demonstrated that intellectual competence cannot be accurately represented by a simple IQ score, or measured solely by tests of verbal, mathematical, or so-called nonverbal abilities, important as these are. Learning and cognitive development in children as reflected in the child's performance also embraces such other dimensions of intelligence as originality, flexibility, and creativity. The Growth and Development Branch continues to seek new and better ways to understand and explain these elusive qualities which are so difficult to measure; considerable developmental work is currently underway at major laboratories throughout the country to more reliably define and assess them.

During this reporting period there has been a growing interest among researchers in how children develop the language capabilities that they must later use as adults in speaking and in reading. In the human communication area, recently reported research with very young infants may well open the way to direct investigations of certain innate versus acquired abilities. For example, one grantee has found that one- and four- month old infants are able to discriminate the acoustic cue underlying the adult phonetic distinction between the consonants "b" and "p". Moreover, and of even more importance, these researchers have found there was a tendency in these subjects toward categorical perception, i.e., the manner in which adults perceive these same sounds. This may prove to be the first evidence that

perception, in a linguistic mode, may well be part of the biological make-up of the organism and are operative at an unexpectedly early age.

Adult Development and Aging Branch

If atherosclerosis and cancer are brought largely under control, as now seems probable, what will be the health status of the elderly spared those diseases at least until much later in life? The answer to this question lies in the way control over disease is achieved. The causes of disease can be placed in three categories. One category then contains environmental factors--nutrition, infection, etc. Another category contains a host of genetic defects. The third category contains intrinsic aging processes. The nation is gaining control over its health problems by modification of environmental factors. It is beginning to see hope for the control of genetic factors. However, little progress has been made in the understanding of aging processes and even less in the development of methods for mitigating those processes. Aging processes are important in the origin of many disorders: coronary, cerebral, and other atherosclerosis, many types of cancer, some types of diabetes mellitus, osteoporosis, osteoarthritis, presbyopia, cataracts, glaucoma precipitated by lenticular enlargement, vitreal degeneration precipitating retinal detachment, macular degeneration of retina, presbycusis, senile dementia, emphysema, ovarian failure resulting in the menopause, and impaired resistence to infectious diseases. All of the biologic studies in the Adult Development and Aging Branch bear on the deteriorations of function and thus the development of disease with age.

The poor resistance of the elderly to infection is well known and probably due in part to a reduction in the efficacy of the system controlling circulating antibodies. Less well known is the decrease in cellular immunity with age. This can be shown experimentally by the decrease in the ability of the old animal to reject transplants of tissue from antigenically different animals. It turns out that this decrease in cellular immunity may be important in the great increase in the occurrence of cancer in the elderly. The immune system becomes unable to destroy the initially small groups of malignant cells that probably come into existence over much of the life-span and thus permits them to grow into clinically important cancers. The Institute supports some work on the decay in immunologic competence with age.

Mental Retardation Branch

The greatest hope for mastery of the problem of mental retardation lies in its prevention, or when this is not possible, in the promotion of the individual's maximum skills through a wide range of habilitative and restorative procedures. The goals of the Mental Retardation Branch, then, are the facilitation of research directed toward the identification, prevention, correction and amelioration of mental retardation. Typical of the work supported by the Branch is that described below.

At the University of Washington, investigators are engeged in intensive studies of the activity of neurons generating the epileptic process in animal models and in humans. The mechanisms by which the abnormal firing of neurons is propagated throughout the nervous system at intervals can now be identified

and the synaptic mechanisms involved are clear. The most common cause of the hyperactivity is neonatal or acquired injury. This often stimulates altered synaptic connections with possible abnormal circuit action behaviorally reflected as mental retardation. Knowledge of these processes is leading to new types of therapy. One form, involving the production of subcortical lesions, has been markedly effective in reducing seizures in monkeys. These same animals are also being used to test new anticonvulsant drugs.

The impact of psychosocial deprivation on the intellectual development of infants and very young children is being actively studied by Center investigators at the University of Wisconsin. A group of infants, whose mothers and older siblings are characterized by subnormal levels of intellectual performance, minority group status and socioeconomic deprivation, have been enrolled in an enriched program of mental stimulation and socialization. The mothers have also received guidance in child-rearing and training for vocational placement. At the age of three years, these children are significantly ahead of a matched control group in mental, social and personality development. They will continue to be tested to determine whether these gains will be sustained through school age.

In the six years since the Mental Retardation Research Centers program was first initiated, twelve centers have been created. Eleven are located on university campuses while the twelfth is closely affiliated with several universities and is on the site of a state institution. Construction has been completed at all but two of the centers (Wisconsin and North Carolina) and at one part of the three site complex at Kansas.

The research and training activities of the centers are highly varied and embrace basic, clinical, and applied approaches in the biomedical, behavioral and social aspects of mental retardation and related aspects of human development.

One element of the Centers program recognizes the importance of early detection and diagnosis to prevention and amelioration of mental retardation. An epidemiological study of a total birth cohort in a community has already been completed by researchers at Einstein Medical College, resulting in a better appreciation of incidence and prevalence and the biological and socioenvironmental variables with which mental retardation is associated.

Again, a fairly reliable screening instrument for detection of abnormalities which can be effectively administered by nonprofessional personnel is being developed by investigators at Colorado. The ability to identify large numbers of children with subtle deficits early in life will permit intervention during crucial phases of growth. Other efforts in these general areas include profiles and population mobility studies in institutions; relationship of high risk factors to learning disability; diagnosis of minimal brain damage; effect of alternative patterns of care on behavior and development.

The highly practical goals of the Research Centers program however, cannot be achieved without the substrate of fundamental knowledge of biology the molecular level. Studies of neural tissues, embryonic development, genetic mechanisms, cell growth and fetal endocrinology are examples of such research

by Center investigators. While less immediate to the problem of mental retardation, the fundamental knowledge to be derived from these areas of study form the bridges to ultimate prevention.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Office of the Associate Director for Program Planning and Evaluation

The Associate Director for Program Planning and Evaluation and his staff are responsible for developing the information needed for the planning and the formulation of the Institute's major goals and objectives and the means for appraising progress toward these objectives.

In performing these functions, the Office: (1) sponsors and conducts studies on the nature of the emerging problems and needs within the broad field of child health and human development; (2) stimulates and provides guidance for the analysis of the status, character, and extent of scientific activity in problem areas and the resources needed for further Institute efforts; (3) coordinates activities leading to the selection of Institute objectives and the development of program plans; (4) prepares and maintains a comprehensive multi-year program plan utilizing operational planning systems and other analytical techniques; (5) sponsors and conducts reviews of program activities to assess progress toward objectives and to evaluate accomplishments, and (6) maintains an awareness of ongoing Congressional activities, keeps staff informed of legislative developments, and coordinates matters of mutual concern with other interested Governmental and non-Governmental organizations.

Dr. Sam Greenhouse is Acting Associate Director of the Office of Program Planning and Evaluation of the NICHD. His staff includes an Assistant Director for Clinical Programs, Facilities, and Resources; an Assistant Director for the Behavioral Sciences; an Assistant Director for the Biological Sciences; a Program Analyst, a Legislative Reference Assistant, and supporting secretarial staff.

An important continuing activity of the Office is an assessment and review of past NICHD activities in the effort to provide a framework for formulating long-range goals in terms of the Institute's mission and the needs of the '70s and '80s. The basic issue relates to the Institute's orientation, focus, and programmatic directions that will lead to achievements and directions relevant to the world of 1980 and, again, of 1990. The aim is to provide alternative goals and objectives that remain flexible and that are subject to continuing review and modification in terms of the needs of the time.

In Fiscal Year 1971, the Office of Program Planning and Evaluation prepared several reports on aspects of the research and training programs of the Institute in response to requests from Congressional Committees and for inter-agency coordination purposes. There has been much interest in how Institute research and training programs pertaining to early child development interface with other Federal efforts in this area. Several pieces of legislation concerned with Federal support of research and services specifically aimed at improving the health status of children have been introduced in the Congress. All of these bills have some impact on NICHD research activities.

Staff of OPPE, in cooperation with the Budget Officer and Executive Officer of the Institute, developed the Program Financial Plan for FY 1972-76 and the Operational Plan for FY 1971.

A Conference on Research in Child Development and the Man-Made Environment (April 1971) was developed cooperatively by staff of OPPE and the Office of the Director. The aim of this conference was to determine and define (1) those factors in the environment which have serious impact on child development and (2) the research necessary to relate changes contemplated in the built environment to optimal child development.

The death of infants suddenly from unknown cause accounts for about one-tenth of the total infant mortality in the United States. OPPE staff have initiated cooperative efforts to expand research concerned with the causes and prevention of Sudden Infant Death Syndrome (SIDS). These efforts include staff of the Epidemiology and Biostatistics Sections, of the Epidemiology and Biometry Branch and the PBIM Branch, Extramural Programs. This team approach to an important pediatric problem includes development of collaborative research projects and the definition of research goals and needs as well as the coordination of Institute research efforts in this area.

In the past century the number of medications administered to pregnant women has multiplied enormously. It is possible that at present the fetus is potentially at greater risk from the well-intentioned use of pharmaceuticals than from the stress of labor and delivery. Staff of OPPE, in collaboration with staff of the PBIM Branch are cooperating to develop a research focus that is concerned with effects of medications administered during pregnancy and childbirth on the developing infants' behavioral and physiological functioning.

The OPPE staff is also involved in contract negotiations with the Social Science Research Council for a study entitled "Genetic Factors in Human Performance," together with the National Institute of Mental Health and the Office of Child Development.

The facilities and resources activity of the OPPE has included the function of coordinator of planning for Building 33, the NICHD Research Facility to be built on the NIH campus. This building, now in final stages of design, will contain approximately 90,000 net square feet. It also includes participation in the development of plans for an intramural clinical research unit to be activated in the Clinical Center, NIH, via an addition of 15,492 net square feet to be made available through construction of an 8th, 9th, and 10th floor on wing G. Target date for occupancy is January 1, 1973.

OPPE staff has participated in coordination efforts, and maintains liaison, with a sizable number of agencies. These include the Office of Child Development DHEW Planning Team on Provision of Health Services for Mothers and Children, DHEW Secretary's Advisory Committee on Health Protection and Disease, Council on Child Health of the American Academy of Pediatrics and its Accident Prevention Committee, the Interdepartmental Committee on Children and Youth, the Community Coordinated Child Care (4-C) Standing Committee, the White House Conference on Children and Youth, the Interagency Committee on Early

Child Development, the Task Force on Child Advocacy (to implement the recommendations of the Joint Commission on Mental Health of Children), and a committee on policy for the American Council on Education. The OPPE staff serve as Chairman of the NICHD Safety Committee, and as Chairman and members of the NICHD Equal Employment Opportunity Committee, and as Chairman of the new NICHD Scientific Publications Steering Committee.

The Acting Associate Director is representing the Institute at the NIH Advisory Committee for Program Planning and Evaluation. Dr. Greenhouse was also asked to be Chairman of the Evaluation Sub-Committee of the parent Advisory Committee and has served in this capacity since becoming Acting Associate Director. The Sub-Committee is responsible for developing policy on evaluation at the NIH and serving as an advisory group to review evaluation plans, to develop a framework for establishing priorities of programs to be evaluated and to advise on the design of evaluation studies.

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Office of Public Information

At the conclusion of fiscal year 1971, the Public Information Office was realigning office responsibilities and emphases to maximize the effectiveness of the present staff. Based on three years experience at the Gerontology Research Center in Baltimore and two years' experience with the Center for Population Research, it was evident that organizing information activities around unit or program activities made for effective staff use. Such an organization, it is felt, is particularly useful in NICHD, which has a wide and highly disparate group of programs. Each of these must be intimately known by public information specialists if they are to receive appropriate recognition in the media, and within the Institute's various publics.

Information activities at the Gerontology Research Center, which has had the exclusive services of a public information officer for the past three years, were numerous. The Center Chief and Dr. Reubin Andres discussed aging research on "You and Everyone's Future," shown twice over WRC-TV (Washington) in early 1971. The Information Officer wrote the working script and helped plan this HEW-produced program. Other television outlets served were Westinghouse Broadcasting, West German Television, National Educational Television, WJZ-TV (Baltimore), and the Maryland Center for Public Broadcasting. Popular articles on the Center appeared in The New York Times, Saturday Review, True, Family Weekly, Good Housekeeping, The Baltimore Sun, and the Baltimore News-American. Contacts were also made with Newsweek, The Wall Street Journal, The National Observer, and The Philadelphia Inquirer. Specialized publications requesting information included MD Medical News, Today's Health, Modern Maturity, Health, and Bell Labs News. Foreign media interest was evident through contacts with Mainichi Newspapers (Japan), Der Spiegel (West Germany), UPI (London), the French News Agency, and Editorial Exito, S.A. (Spain).

The number of Center tours increased to 38 this year from 24 last year (up 58%), and inquiries from the public rose to 193 from 162 (up 19%). Tours or briefings were held for groups ranging from high school students to foreign scientists. In addition, special Baltimore itineraries were set up for visitors from Belgium and Wales.

Other activities included editing or rewriting a number of scientific papers for publication in the Gerontologist, and writing an article on studies in the Laboratory of Cellular and Comparative Physiology for Zeiss Information.

The Center for Population Research has had a public information specialist assigned to it for the past two years. This has resulted in the close integration of program and public information activities during a period of exceedingly rapid growth within the Center.

Highlights of public information activities last year included the issuance of a pamphlet, Population Research, covering all of the Institute's population activities. Nearly 9,000 copies of the pamphlet have been distributed,

and its excellence is such that it was awarded a Blue Pencil Award in the Federal Editor's Association annual publications contest.

Public and press inquiries to the Center almost doubled during the year, resulting in an increasingly heavy work load. Major press activities included an October 1 press briefing held at the conclusion of an International Conference on "Regulation of Mammalian Reproduction," sponsored jointly with the Fogarty International Center. The briefing was attended by approximately 15 press and television reporters. Interviews with CPR staff have been arranged for Associated Press, Wall Street Journal, New York Times, Biomedical News, Medical World News, etc.

In addition to media activities, the public information specialist assigned to the CPR was responsible for drafting or editing several reports on CPR programs for the Office of the Secretary, DHEW, and other units of the executive branch. She also edited two books for publication by an outside publisher.

Audio-Visual Activities

Three major network shows featured NICHD activities. With NICHD program staff functioning as consultants, Time-Life International produced "Rock-A-Bye Baby," which was shown on Channel 26's "The World We Live In" series. Marion Sanders of ABC-TV interviewed three Institute scientists for a network show on population problems, and NBC's documentary, "Childhood, The Enchanted Years," featured NICHD supported investigators. PIO staff contributed liaison services to each of the latter two programs.

In addition, full use was made of Department sponsored radio and television programs, resulting in the radio or television appearance of Drs. LaVeck, Lowe, Lipsett, Corfman, Tjossem, Hasselmeyer, Shock, Andres, and Mr. Jehu Hunter.

Publications Activities

With the transference of conference publications responsibilities to the Scientific Information Centers Branch just prior to the beginning of the fiscal year, the scope of PIO involvement in overall Institute publications was reduced.

However, the last fiscal year did see the production and distribution of several noteworthy volumes. Besides the pamphlet on "Population Research," which was mentioned earlier, the Office edited and distributed the long-awaited volume "Research Progress, 1963-1970," which describes the scope of Institute programs and outlines progress made in the various fields by Institute supported investigators.

Late in the fiscal year, the 544-page conference proceedings "Secretory Immunologic System," begun the previous year, was issued. The PIO's technical editor was responsible for the editing, printing, and distribution aspects of this outstanding volume. In addition, she compiled a bibliography for the publication of over 600 references.

Other publications and editing activities included compilation and editing of a new edition of Who's Who in NICHD, the coordination and compilation of the Annual Report, approximately 1000 pages, and the compilation of material for the Scientific Directory and Annual Bibliography.

It is worth noting that all of these tasks, plus the editing of "Secretory Immunologic Systems," were the responsibility of the Technical Editor--a very heavy work load. In addition, she coordinated work with medical arts on the cover design for Issues in Human Development, arranged for a second printing of the Bibliography on Death and Dying, revised and designed the form for NICHD speech and scientific papers clearance, and initiated a new procedure for record-keeping of NICHD scientific articles retrieved as a result of journal screening.

Summary of Activities

Following a rapid growth in the number of press and public inquiries handled by the Public Information Office in the last few years, fiscal year 1970-71 saw a levelling off of these inquiries. Approximately 250 press inquiries from about 90 media were handled, and approximately 8,000 public inquiries. About 85,000 publications were distributed, over 100 features and press releases prepared, 19 radio and television appearances arranged for, 111 Congressional inquiries responsed too. Screening of approximately 350 scientific journals throughout the year resulted in the identification of approximately 1,500 scientific articles published by NICHD grantees and staff. NEWS FROM NICHD was prepared and mailed twice during the year to over 360 scientific journals, and the public relations offices of grantee institutions were notified concerning the awarding of approximately 200 research and training grants. Approximately 332 scientific articles were cleared.

Present Problems and Future Plans

Problems outlined in the last annual report continued through the year. Despite increases in the Institute programs and budget, the Office of Public Information has the same number of staff at the conclusion of fiscal 1971 as it had at the end of 1965.

Thus effective function requires careful evaluation of each of the office's activities if the Institute's activities are to be well represented in the press.

The new realignment of office responsibilities and emphases should result in careful planning with Institute program staff concerning necessary and effective information activities. Of prime importance will be the careful development of sound information activities related to the Institute's intramural programs.

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NEWS CONTACTS
Fiscal 1971

Alabama Journal
American Chemical Society
News Service
Annals of Internal Medicine
Associated Press

Baltimore Evening Sun
Baltimore News American
Behavior Today
Bell Laboratory News
Blue Sheet

Chemical Engineering News
Changing Times
Chicago News Service
Chicago Sun Times
Chicago Tribune
Cox Newspapers

Der Spiegel
Drug Topics Red Book

Fairchild Publications
Family Health
Family Weekly

Globe Newspapers
Good Housekeeping

Harvest Years
Hospital Practice
Hospital Topics

Jack Anderson Column
JAMA
Jefferson Standard Broadcasting Co.
Journal Herald, Dayton
Journal of Nutrition Education

Knight Newspapers

Life
London Bureau, UPI
London Daily Express

Mainchichi Newspapers

McCalls
MD Medical News Magazine
Medical Economics
Medical Opinion
Medical Tribune
Medical World News
Medill Publications
Miami Herald
Minneapolis Tribune
Missourian
Mother Earth News

National Enquirer
National Geographic
National Journal
National Observer
Natural History
The New Scholar
New York Times
Newsweek

Pediatric Herald
Pediatric News
Philadelphia Inquirer
Plenum Publishing Corporation
Popular Mechanics
Psychology Today

Readers' Digest
RN Magazine

Saturday Review
Science World
Scripps-Howard
Statistical Bulletin
Stromberg Publications

Time Magazine
Today's Health
Trial Magazine
True

United Press International
US Medicine

Wall Street Journal
Washington Evening Star
Washington Post

Audio-Visual Contacts

ABC-TV News
CBS-News
Jaffe Stivers Productions
Jefferson Standard Broadcasting Co.
National Presbyterian Center
NET-TV
NBC
Panarama
Public Broadcasting Service
RAI Italian Radio-TV
Voice of America
WBAL-TV Baltimore
WBJC Baltimore
Westinghouse Broadcasting
WJZ-TV
WSJ
WTTG

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Epidemiology & Biometry Branch

The Epidemiology and Biometry Branch is primarily concerned with 1) carrying out epidemiologic studies in areas related to the mission of the Institute; 2) providing consultation in statistics and mathematics to scientists in both the intramural and extramural programs of the Institute; 3) and performing research in statistical theory and methodology. The Branch assumes the major responsibility within the NICHD for the validity of design and methodology and for the most appropriate techniques available for analyzing data in laboratory experiments and in clinical and community studies. The Branch is also responsible for the periodic analysis of basic vital statistics and other demographic data to determine essential characteristics of basic series related to the health of the Nation such as births and birth weight, infant mortality, and population distribution.

In June 1970, two sections were formally established within the Branch: a Biostatistics Section and an Epidemiology Section. This organization reflected the increasing functions and activities in each of these two major areas of responsibility. The Biostatistics Section will, for the present, assume the mathematical statistical activities of the Branch such as providing consultation in the design and analysis of experiments and in carrying on research in statistical methods. The annual reports of the Sections are presented separately.

The Branch has been involved in two significant developments concerning registries. One concerns the technique of amniocentesis which is being used more and more frequently to monitor the fetus of a high risk mother. The Scientific Director of the NICHD initiated a series of meetings with leading practitioners of the procedure at which members of the Branch were present to aid in the planning of the establishment of a central registry to assess the risk of the technique. The Branch has assumed the responsibility for maintaining this registry and for the analysis of the data. The Branch has also given very serious consideration to undertaking a similar role with regard to a birth defects registry. Members of the Branch have been in contact with investigators who maintain such registries to explore the need for a coordinated effort on a national basis. The NICHD is extremely interested in determining whether a group of such registries representing different regions of the United States can serve as a surveillance device for detecting teratogenic environmental agents and, secondly, whether the information gathered by registries constitutes a useful data base for specific research studies.

Activities of the Branch

During the year, the Branch Chief continued to serve on the:

FDA Biometry and Epidemiology Advisory Committee
NCI Biometry and Epidemiology Contract Review Committee
HEW Career Service Board for Mathematicians and Statisticians

He was appointed to serve a 3-year term on the National Research Council, Division of Biology and Agriculture, as a representative of The Biometric Society. He also is a member of the Epidemiology and Statistics Advisory Committee of the Perinatal Research Branch, NINDS.

The Branch Chief presented two invited lectures on Statistical Problems in Bio-Medical Research: one, at the Johns Hopkins University and the other at The George Washington University.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Epidemiology & Biometry Branch
Epidemiology Section

Research Activities

1. Dr. Stark served as Project Officer on 3 research contracts:

   a. Intravascular Coagulation in Respiratory Distress Syndrome with Georgetown University.

   b. Detailed Data for Over 2 Million Births (January 1966 - June 1968) with Commission on Professional and Hospital Activities.

   c. Relative Efficacy of Two Different Prenatal Care Regimens with Contra Costa County Medical Services.

   See the contract reports for details about these studies.

2. See Item 2 under "Research Activities" in Dr. Seigel's 70-71 Annual Report.

3. Dr. Stark reviewed all literature concerned with the epidemiology of Respiratory Distress Syndrome of the Newborn. A manuscript for this review was submitted to "Pediatrics" and it was rejected. A revised manuscript will be submitted to "Biology of the Neonate".

4. Dr. Rudzki reviewed all literature concerned with the epidemiology of mental retardation. The purpose of this review was to provide a basis for planning future section activities in this area. This review has 8 specific goals:

   a. To obtain information regarding prevalence and incidence of this syndrome in different populations. To ascertain the factors that affect the wide variation in MR prevalence rates reported in these studies.

   b. To analyse the proportion of organic vs "non organic" MR that contributes to the total number, and to determine to what extent each of these 2 categories is responsible for the different subtypes according to IQ and other parameters such as presence or absence of pathologic neurological signs.

   c. To determine the consistency of the published information on the prenatal, natal and postnatal events that might affect the outcome of MR children and critically analyse their contribution to each subtype of MR.

   d. To obtain information from different studies regarding the socio-economic background of families with MR children.

e. To search for parameters that will identify the group or groups at risk of MR in the population.

f. To determine whether the change in social class for Blacks has affected the MR prevalence rate and if so to what extent.

g. To obtain experimental and epidemiologic information on the presence of nutritional deficiencies in mothers and retarded children by subtype vs control population.

h. To search for studies dealing with information on intercurrent events in a cohort of children with decreasing IQ and other achievement scores compared to a sample of children that show no decrease through time.

5. Several smaller studies were initiated and brought to various stages of completion during the year:

a. Dr. Stark (in collaboration with Dr. Greenhouse) initiated a study designed to determine the relative importance of gestational age and birth order in determining the risk of fetal, perinatal, and neonatal mortality. Data from New York City for 1964-1967 will be used in the study, and a cost estimate for the data purchase is currently being obtained.

b. A manuscript describing the status of maternal and newborn coagulation systems at the moment of parturition has been completed and will be submitted for publication.

c. A study of the association between birthweight and risk of childhood leukemia is underway. Birthweights of 705 Michigan childhood leukemia deaths are being compared with statewide birthweight distributions to test the hypothesis that birthweights of leukemic children are higher than the general population. This study will be completed by June 30, 1971.

d. A study of the maternal-age effect on the risk of Down's Syndrome in Nonwhites is also underway, and will be completed by June 30, 1971. This will be the largest American study to date and it should give a definitive answer to the question of whether maternal-age effects are different in White and Nonwhites.

e. Negotiations with NICHS to obtain the U.S. birth-abstract tapes for 1968 are well along. We expect a cost estimate soon, and as soon as the tapes are received work will begin on a descriptive study of low-weight-for-gestational-age babies. U.S. LWA rates will be examined by race, sex, maternal age, parity, month of birth, and place of birth. If geographic variation is marked, we will attempt to demonstrate a correlation between LWA rates and data from the National Nutrition Survey.

f. Data has already been acquired for a study of RDS and prematurity in over 50,000 births to American Indians. This data will permit us to determine RDS risk by MA, parity, birthweight, and tribal origin. Data analysis will commence in the summer of 1971.

Consultation

1. The Brewer study, mentioned elsewhere, grew out of a consultation with Dr. Lowe in the intramural area. As well as undertaking the Brewer contract, we are helping Dr. Lowe to plan additional studies of maternal nutrition during gestation and its effect on the outcome of pregnancy.

2. An effort to develop a closer working relationship with the Perinatal Biology and Infant Mortality Branch has resulted in an active consultation. The Epidemiology Section will work closely with PBIM to develop a new program concerned with clinical and epidemiologic aspects of narcotic addiction in pregnant women and newborns. The first contract of this program should be funded before the end of FY 71.

3. An effort to develop a closer working relationship with the Growth and Development Branch has not yet resulted in any specific consultations. However, Dr. Stark will join Dr. Read in a visit to Mrs. Agnes Higgins of Montreal, Canada, in June of 1971, and this visit could develop into a productive consultation.

Other Activities

1. Dr. Stark has continued his teaching activities in the Department of Pediatrics at the Georgetown University Hospital. For the month of April 1971, he was attending physician for the GUH nurseries.

2. During the year Dr. Rudzki passed the "oral" examination for the American Board of Internal Medicine, and he is now a fully certified diplomate.

3. During the months of August and September, 1970, Dr. Rudzki acted as attending physician in internal medicine at the Washington VA Hospital.

Bibliography

Papers:

1. Stark, C.R., Niswander, J.D., Bardanouve, V.T., and Iba, B.: Temporal-Spatial Clustering of Oral-Cleft Births in Michigan and Montana. Cleft Palate Journal 7:826-845, October 1970.

2. Rudzki, C., Ishak, K., Katz, S., and Zimmerman, H.J.: Chronic Intrahepatic Cholestasis in sarcoidosis. Clinical Research XVIII:388, 1970. (Abstract)

Letter:

1. Stark, C.R., and Rudzki, C. (letter): Infectious hepatitis and Down's Syndrome. Lancet 2:572-573, September 1970.

Talks:

1. Stark, C.R., Abramson, D.C., Erkan, N.V., and White, N.B.: Status of Maternal and newborn coagulation systems at the moment of parturition. Presented at the Sixth Joint Meeting of the Clinical Society and Commissioned Officers Association of the U.S. Public Health Service, April 4-7, 1971, Galveston, Texas.

2. Stark, C.R., Niswander, J.D., Bardanouve, V.T., and Iba, B.: Temporal-Spatial Clustering of Oral-Cleft Births in Michigan and Montana. Presented at the Sixth Joint Meeting of the Clinical Society and Commissioned Officers Association of the U.S. Public Health Service, April 4-7, 1971, Galveston, Texas.

3. Rudzki, C., Ishak, K., Katz, S., and Zimmerman, H.J.: Chronic intrahepatic cholestasis in sarcoidosis. Presented at the Sixth Joint Meeting of the Clinical Society and Commissioned Officers Association of the U.S. Public Health Service, April 4-7, 1971, Galveston, Texas.

Contract Title: Detailed Record Abstracts for Over 2 Million Births Over a 2½ Year Period.
Contractor: Commission on Professional and Hospital Activities
Money Allocated: $8,419

Objective:

To create a table of 2,056,467 U.S. births. The table dimensions will include: live or dead at discharge, premature-non premature, ≤2500 grams or >2500 grams, RDS or not RDS, male or female, racial origin, method of bill payment, U.S. census region, and month of birth.

Summary: All birth records that entered the CPHA-PAS system between January 1966, and June 1968, will be tabled. Both a magnetic tape and printed output will be provided to the Epidemiology Section of the E & B Branch of NICHD for analysis.

Significance to Biomedical Research and Program of the Institute: The data will provide a basis for determining any associations between risk of RDS and risk of death before discharge from the nursery on the one hand and all of the classification variables on the other. For the first time ever it will be possible to determine whether RDS and newborn deaths follow a seasonal pattern of occurrence.

Progress in the First Year: The data has been delivered and analysis has commenced. No further data acquisitions are planned at this time.

Proposed Course: Data analysis will be completed in the summer of 1971, and a manuscript written soon thereafter.

Contract Title: Relative Efficacy of Two Different Prenatal Care Regimens
Contractor: Contra Costa County Medical Services
Money Allocated: $50,000

Objectives:

1) Contract: to supply NICHD with sufficient data to make the desired tests of hypotheses

2) Study: to determine whether pregnant women treated with nutrition lectures, no weight control, and no drugs bear healthier babies than women treated with a traditional regimen of weight restriction, diuretics, and low calorie-low salt diets.

Summary: The contractor will abstract about 7,200 case records and code the abstracted material. Code sheets will be forwarded to NICHD for processing and analysis. The Epidemiology Section of the E & B Branch will perform analyses to achieve the study objective.

Significance to Biomedical Research and Program of the Institute: It is anticipated that this study will show that avoidance of gestational weight control, avoidance of drugs, and special attention to nutrition will result in a low-birth-weight rate of about 3% and a complete absence of the "toxemia of pregnancy" syndrome. This result will enable the Institute to plan and execute subsequent studies which will form a basis for recommendations which will ultimately lead to considerable reductions in U.S. infant mortality.

Progress in First Year: It is expected that the contractor will attain his objective within a 1 year period.

Proposed Course: It is expected that data analysis will be completed during the summer of 1972.

Contract Title: Intravascular Coagulation in Respiratory Distress Syndrome
Contractor: Georgetown University, Department of Pediatrics
Money Allocated: $63,634

Objectives:

Primary 1) To determine whether DIC occurs in infants with RDS.

2) To determine the extent of intravascular coagulation in premature infants with and without RDS.

3) To determine the extent of intravascular coagulation in premature infants who succumb to and survive RDS.

Secondary To establish norms for coagulation factors and fibrinolytic activity in premature infants by age in hours.

Summary: Data on the coagulation-fibrinolysis status of about 1000 premature infants and their mothers is to be collected at Georgetown University Hospital and 4 affiliated hospitals. This data will be used to establish a set of coagulation-fibrinolysis norms for the perinatal period and to test the hypotheses listed in "Objectives" above.

Significance to Biomedical Research and Program of the Institute: This study will provide a partial test of the theory which holds that at least one cause of the respiratory distress syndrome is intrapartum disseminated intravascular coagulation. If the study supports the theory, many new approaches to therapy of RDS will be possible.

Progress in the First Year: During the first year of operation the contractor established a coagulation laboratory, set up a specimen collection system, collected sufficient coagulation data on mothers and newborns at the time of delivery to warrant 2 publications, and he collected coagulation data which will be used to test the hypotheses listed in "Objectives" above.

Proposed Course: Because of numerous extenuating circumstances, data acquisition is considerably behind the originally proposed schedule. During the first (and last) renewal year the contractor will endeavor to collect sufficient data to test the study hypotheses. If the data provide support for the theory that disseminated intravascular coagulation is involved in RDS etiology, the contractor will seek a new contract or grant to test the efficacy of heparin therapy for RDS.

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Epidemiology & Biometry Branch
Biostatistics Section

Research Activities - Health Studies

1. One statistician is responsible for a registry of amniocentesis in eight laboratories in the United States which is being developed. She coordinates the data from these centers and analyzes them to evaluate both the efficacy and the risk of the procedure. She has been active in designing the study and developing systems for data processing.

2. A study is being developed in military hospitals to determine whether a history of oral contraceptive use is associated with an increased risk of foetal loss during pregnancy or of infant morbidity in the liveborn. This study is scheduled to be under way by the summer of 1971. At the same time national statistics on mortality from congenital malformations, and selected series of morbidity from such diseases are being analyzed to detect changes in trends during the years that the oral contraceptive came into use.

3. A conference was held to advise the Institute on the need for a birth defect reporting system which would be used to monitor the hazards of environmental agents that manifest themselves through foetal damage. If the recommendation is made that such a system is required the Section is prepared to develop a research proposal.

4. Section personnel serve as Project Officers on five research contracts:

a) A case-control study of carcinoma and oral contraceptive use in women of reproductive ages in the State of California.

b) A case-control study of carcinoma and oral contraceptive use in women in the reproductive ages in the State of Connecticut.

c) A collaborative case-control study of stroke and oral contraceptive use in women in the reproductive ages in several hospitals.

d) A study of the correlates of intrauterine device effectiveness in family planning clinics in California.

e) A study in Yugoslavia of the risks associated with induced abortions, supported through P. L. 480 funds.

Research Activities - Statistical Methods

1. One member of the Section has been working on the problem of transformations in the analysis of variance. A manuscript has been submitted for publication as an outgrowth of this work.

2. A member of the Section is especially interested in time series analysis and is working on the problem of robust spectrum estimation.

3. A study is being made of the circumstances in which matching is appropriate in case-control studies and the effect upon estimates of relative risk of inappropriate matching.

4. A study was done of the ability of three epidemiologic instruments to detect increased risk of disease resulting from a suspected agent. The methods compared were the case-control, the cohort, and the historical analysis. The results of this analysis have been sumbitted for publication.

Consultation

1. One member of the staff has been entirely responsible for statistical consultation in the Gerontology Research Center (GRC). This includes developing the format of routine statistical analyses of the longitudinal study, coordinating programming within DCRT with the scientific activities of investigators at the GRC, and consulting on technical statistical problems with individual investigators.

2. Another member of the Section is providing consultative support to the Center for Population Research, particularly in studies of the medical effects of contraceptives. Considerable time was spent in developing and reviewing studies in hypertension and in cervical cancer.

Other Activities

1. Dr. Seigel continues to serve as a consultant to the National Heart and Lung Institute in its joint study with the Veteran's Administration on arthritis and thromboembolism.

2. Dr. Seigel served as a consultant for the WHO, serving on a study group to select suitable sites for the development of centers to conduct trials of contraceptive agents.

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Epidemiology & Biometry Branch
Biostatistics Section

Publications and Talks

1. Seigel, Daniel G.: Oral contraceptives and thromboembolic disorders: Epidemiologic studies. Ann. Inter. Med. 74:258-261, 1971.

2. Seigel, Daniel G.: Venous thrombosis: Pregnancy, puerperium, and the steroid contraceptive. In proceedings of workshop on venous thrombosis sponsored by the National Heart and Lung Institute. In press.

3. Seigel, Daniel G.: A multi-phasic prospective study of women using oral contraceptives. Invited talk at the International Conference on Adverse Reaction Reporting Systems, October 22-23, 1970.

4. Seigel, Daniel G.: The use of historical data and adverse reaction reporting systems for epidemiologic study. Amer. J. Epidem. In press.

5. Schlesselman, James J.: Power families: A note on the Box and Cox transformation. J. Royal Stat. Soc., Series B. In press.

6. Seigel, Daniel G.: Letter to the Editor. New Eng. J. Med. In press.

7. Seigel, Daniel G.: Letter to the Editor. Obstet. Gynec. In press.

NICHD Annual Report
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Epidemiology & Biometry Branch
Biostatistics Section
Contract and Collaborative Research

Contract Title : A Retrospective Study of the Risks for Cancers of the Breast, Body of the Uterus, Ovary, and Cervix Among Users of Oral Contraceptives
Contractor : Yale University, New Haven, Connecticut
Money Allocated : No funds allocated for FY 1971

Objectives: To determine whether the incidence of carcinoma of selected sites is associated with oral contraceptive use.

Summary: Cases are being identified of women with breast, uterine, and ovarian cancer admitted to hospitals in the areas in and around Bridgeport, Hartford, and New Haven, Connecticut, and Springfield, Massachusetts, over a two-year period. A total of 900 such cases is expected between the ages of 20-44.

Controls are selected as the next patient with the appropriate matching variables admitted to the same hospital as the case with an acute medical or surgical condition or for an elective operation.

Interviews are administered in hospital, whenever possible, inquiring of demographic variables, obstetric history, menstrual history, contraceptive history, and a checklist of diseases.

Analysis will involve an examination of the difference in the proportions of cases and controls exposed to oral contraceptives for different lengths of time, for each site and for specific cell-types at each of the four sites. Relative risks for various lengths of exposure to the oral contraceptive will be calculated.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: In the initial year, reporting mechanisms, matching procedures, and questionnaires have been developed. A considerable number of cases have been identified and interviewed. The coming year should provide a sufficient number such that an analysis is completed, a decision will be made as to whether this surveillance system needs to be continued, and if so, at what case finding level.

NICHD Annual Report
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Epidemiology & Biometry Branch
Biostatistics Section
Contract and Collaborative Research

Contract Title : A Collaborative Study of Oral Contraception and Cerebrovascular Disease
Contractor : Duke University Medical Center
Money Allocated : $50,358

Objectives: To determine whether the incidence of stroke in young women is increased by use of oral contraceptives, and if so to identify characteristics that are especially related.

Summary: During a two-year period the Neurologic Services of ten university medical centers have identified all young women admitted to their hospitals with definite or suspected cerebrovascular disease of any type. Four of the participating hospitals also identify patients admitted to each of the community hospitals in their metropolitan areas (Miami, St. Louis, Baltimore, and Atlanta). An estimated 200 patients with cerebrovascular disease are submitted for study each year. Clinical data on each of these patients are abstracted from the hospital and physician's records. The frequency of usage of oral contraception in these patients will be compared with that found in two selected control samples: 1) women hospitalized for other diseases and 2) women representing a "healthy" population residing in the same neighborhood as the patients selected for the study. Information regarding pill usage will be obtained from the patient and control subjects by personal interviews carried out in their homes.

Almost all patients and controls have been selected and interviewed in this year. All clinical data have been assembled at Johns Hopkins, where a coding system has been developed. The final year will be spent in data processing, analysis, and development of a report of the study.

Significance to Biomedical Research and Program of the Institute: The study is an extendion of previous research on thromboembolism and oral contraceptive use in which very few stroke cases were included and is a part of our program to monitor the significant effects on health of currently used contraceptives.

Proposed Course: All aspects of the study should be completed by the summer of 1972.

NICHD Annual Report
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Epidemiology & Biometry Branch
Biostatistics Section
Contract and Collaborative Research

Contract Title : Study of Correlates of Intrauterine Contraceptive Device Effectiveness
Contractor : University of California, Berkeley School of Public Health
Money Allocated : Extended without further funds

Objectives: The aim of the study is to search for physiological, attitudinal and demographic factors which are associated with effectiveness and acceptability of the IUD. The approach is to obtain information through standardized procedures on women provided with IUDs at public family planning clinics throughout California.

Summary: Dr. Albert Pierce was appointed as the new project director for this study in September of 1969. Dr. Pierce is on leave of absence from San Fernando Valley State College, where he is Professor of Sociology. He will be with the study through the final funding year. His task is to complete the last phase of the study, closing out of the data collection, analysis, and reporting of the results. The data consist of information collected on approximately 9,000 women provided with IUDs in 11 county health department and hospital clinics throughout California.

Significance to Biomedical Research and Program of the Institute: This is an area of increasing importance as IUDs are increasingly looked to as an alternative to oral contraceptives.

Proposed Course: The study has been extended without further funding. A final report is being prepared for publication which will be available this coming year.

NICHD Annual Report
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Epidemiology & Biometry Branch
Biostatistics Section
Contract and Collaborative Research

Contract Title : Oral Contraceptives and Tumors of the Breast
Contractor : University of California School of Public Health
Money Allocated : $74,506

Objectives: To determine whether the incidence of tumors of the breast is associated with oral contraceptive use.

Summary: The frequency of oral contraceptive use in women with breast cancer and women with benign breast lesions will be compared with that of control groups of women free of cancer and/or breast lesions. Cases diagnosed in a two-year period will be selected.

Three groups of women are being used:

1) Women with breast cancer 49 years of age or younger in whom cancer of the breast has been diagnosed for the first time during the study period. These are patients admitted consecutively to certain designated hospitals in San Francisco Bay Area.

2) Women with benign breast lesions. These are women biopsied or operated for benign lesions of the breast in the same hospitals and within a specified period of the cancer cases. One woman with a benign breast lesion is matched with each cancer by age, by race, by religion, and by socio-economic status.

3) Women without evidence of breast lesions or cancer of any site. These women serve as controls. Two controls are chosen for each cancer case, one from surgical and one from the medical service.

The aim is to accumulate at least 400 cases in each of the study groups, women with breast cancer, with benign breast lesions, medical controls and surgical controls.

Basic data necessary for the study are derived from two main sources -- review of the hospital record of each subject and a personal interview. The interview seeks information on socio-demographic, menstrual and reproductive and contraceptive variables.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: In the initial year, reporting mechanisms, matching procedures, and questionnaires have been developed. A considerable number of cases have been identified and interviewed. The coming year should provide a

sufficient number such that an analysis of these data can be made. When such an analysis is completed, a decision will be made as whether the surveillance system needs to be continued, and if so, at what case finding level.

NICHD ANNUAL REPORT
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Center for Population Research

FY 1971 was the second full year of operation of the Center for Population Research and the CPR's grant and contract programs supported research and training in a wide range of areas in the biomedical and social sciences. Special emphasis has been given this year to three high priority topics: (1) the development of new contraceptive methods, (2) the evaluation of fertility regulating methods currently in use, and (3) the social science aspects of population research. The number of professionals on the Center's staff increased from fifteen to twenty-two and budget increases for individual activities are indicated in the following table:

	FY 1970 (actual)	FY 1971 (estimate)
Research Contracts		
Contraceptive Development	$3.9 million	$ 6.5 million
Evaluation of Fertility Regulating Methods	1.5	3.1
Social Sciences	1.6	3.5
Research Grants	7.9	10.4
Training	2.7	2.6

The Center's contraceptive development program is one of the most important and visible population research activities currently underway, since the need for an array of new methods of fertility regulation is clearly demonstrated. In November 1970, Dr. Eugenia Rosemberg, who served as a consultant during the previous year, became Chief of the Contraceptive Development Branch, and she has been joined by Drs. Gabriel Bialy, Marvin Karten and Richard Blye. These additional staff members provide the nucleus of the scientific staff required to lead a directed program including product-oriented research and development as well as targeted fundamental research. The Center's initial efforts in contraceptive development consisted of the support of basic research in a limited number of areas in reproductive biology which show special promise for the development of new contraceptive methods. This program is being continued, and in addition, a directed program of more applied research has been launched this year. Contracts have been negotiated for studies on improved techniques of sterilization and improved drug delivery systems, and the initiation of a drug development program involving synthesis, testing, and ultimately, clinical trials of promising new chemical compounds. Pharmaceutical companies have significant contributions to make in the area of drug development, and CPR staff members have therefore explored potential problems relating to patent policies and government-industry relationships; it appears that mutually satisfactory contractual arrangements will be possible with industrial concerns as well as private agencies for the development of new contraceptive agents.

The evaluation of the medical effects of contraceptives presently in use represents a continuation of one of the earliest activities of the CPR. This year we have been fortunate in recruiting Dr. John Schrogie, who previously

was responsible for studies of oral contraceptives at the Food and Drug Administration, to direct this program. A new Fertility Regulating Methods Evaluation Branch has been established and high priority is being given to initiation of biochemical and pharmacological investigations of the oral contraceptives. This program includes studies of metabolism and dose-response relationships as well as biochemical investigations of effects on systems controlling blood pressure and on endocrine function, vitamin and mineral metabolism, and interactions with other drugs. The epidemiological and surveillance studies of oral contraceptive users initiated in previous years are being continued, as are investigations of the mechanism of action of intrauterine devices and the evaluation of techniques for induced abortion. Dr. Helmut Mueller recently joined the staff of this branch, providing additional staff capability for an expanded program.

The Behavioral Sciences Branch directed by Dr. Jerry W. Combs has continued and expanded its programs of contract research in the social sciences with the help of two additional staff members. Dr. Earl Huyck, a sociologist, came to the center after serving as AID's population officer in Costa Rica, and Dr. Duff Gillespie was appointed a staff fellow in sociology. The primary aim of the social science program is the expansion of knowledge of (1) the relationships between population size, growth, composition and distribution and the general welfare or quality of life measured from a variety of points of view, and (2) the social, psychological, economic and other factors which influence birth rates (thus affecting rates of population growth) and migration patterns (which affect population distribution). Areas of emphasis include: (1) the assembly and analysis of data on population trends and the social, economic and cultural contexts in which these occur; (2) the motivations and behaviors related to child bearing which determine the size of completed families and, in interaction with social, economic and cultural influences, help to explain differences in the reproductive behavior of various groups and their variation over time; and (3) the economic, ecological and other consequences of population growth for the individual and the society. A long-range research effort in these areas is foreseen.

The grants program of the Center supports research and training in both reproductive biology and the social sciences. This year saw the initiation of a new program of grants for Population Research Centers which was announced in July 1970. After extensive staff work to inform prospective applicants of the guidelines for these new grants, six applications were reviewed at the March 1971 meeting of the NACHD Council and four more were reviewed in June. Center grants, which provide funds for core services and personnel and for development of new areas of population research in academic and non-profit institutions with established expertise in the population field, will make an important contribution to long-term institutional support for population research. Although funds available for research grants increased in 1971, funds for training grants in the population field shared in the critical scarcity of NIH training funds, and the CPR will be able to fund only one new and two renewal applications of 19 competing applications approved by the reviewing groups. This situation, if continued, will necessarily restrict the needed expansion of research activities in both the biomedical and the social sciences. In January 1971, Dr. James O'Donnell left the CPR staff where he had been Chief of the Population and Reproduction Grants Branch and instrumental in

launching the program of center grants; Dr. O'Donnell left to become the Assistant Director of the NIH Division of Research Resources. The CPR is fortunate to have been able to recruit Dr. Everett Wilson, a reproductive physiologist and Dean of the School of Science at Sam Houston University, as the new Chief of the Grants Branch.

The Center has had a long interest in developing collaborative research projects with foreign scientists under the PL-480 program, and this activity has progressed significantly this year although the development of joint applications involving a foreign investigator and an American colleague, and review procedures in the two countries, are necessarily time consuming. The CPR now supports four active projects; five others which have been approved by NIH await formal approval by the foreign government. Prospects for a considerable expansion of the Center's PL-480 program are indicated by the fact that 39 projects in Egypt, India, Pakistan, Poland and Yugoslavia are now in various stages of preliminary development. A number of these projects are the result of contacts made by members of the CPR staff during travel to PL-480 countries in 1971 and previous years.

The CPR's information and liaison activities with other agencies in the population field have been conducted by the Program Liaison Branch. Dr. Norman Hilmar, Chief of this branch, plans to retire from Federal service on June 30, and at that time the Center expects to transfer these activities to the CPR Office of the Director. An important activity in 1971 was that of the Interagency Committee on Population Research, now a formally established committee. This group has conducted and published an inventory of ongoing Federal support for population research and is now reviewing the data collected and preparing a report for the Office of the Secretary. Dr. Hilmar also served as chairman of the subcommittee on population matters of the FCST ad hoc Committee on Environmental Quality Research and Development. The Program Liaison Branch also was involved with other CPR staff members in preparation of a five-year plan which will be submitted to the Office of the Secretary in accordance with the requirements of the Family Planning Services and Population Research Act of 1970.

In FY 1971, as in previous years, the CPR supported a number of scientific conferences to review current knowledge in areas of its concern. A conference on oogenesis was organized by John D. Biggers and held at Johns Hopkins University in October 1970, and one on the biology of the cervix arranged by Richard J. Blandau will be held near Seattle in June 1971. Dr. Sidney Newman of the Population and Reproduction Grants Branch organized an innovative workshop on research approaches to sex education, contraception, family planning and morality. The CPR joined with the Fogarty International Center to organize and conduct a five day international conference on the regulation of mammalian reproduction which was held at NIH in September 1970. This meeting brought together more than 130 scientists from 23 countries for a comprehensive review of recent developments in reproductive physiology; the proceedings will be published in book form.

Since 1967 the Center for Population Research and its predecessor units in the NICHD have received invaluable advice and guidance in the development of new programs and evaluation of ongoing ones from the Population Research Advisory

Committee, a group of some 17 experts in the biomedical and social science aspects of the population field. In 1971, the Office of the Secretary decided to establish a Secretary's Committee on Population Affairs, with task forces for research and for family planning services to provide the advice required by the CPR and the National Center for Family Planning Services. The Population Research Advisory Committee was therefore disbanded after a final meeting in January 1971. The committee played a major role in the initiation and development of the Center's contract research programs in contraceptive development and the social sciences, and CPR staff members appreciate the time and effort contributed by the committee members. It is anticipated that the new task force may become operational sometime in the next several months to provide continuing advice and guidance from the scientific community.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971
Contraceptive Development Branch

The specific goal of the programs of the Contraceptive Development Branch (CDB) is the development of an array of effective, safe, inexpensive, and reversible contraceptive methods acceptable to various population groups.

During Fiscal Year 1969 and 1970, the Contraceptive Development Program consisted solely of the support through contracts of basic research in reproductive biology. It is well recognized that greater and more intense efforts should be invested in fundamental research concerning the reproductive processes in humans and experimental animals as there are gaps in our knowledge in many areas. However, in order to attain its goal with speed, the Contraceptive Development Branch of the Center for Population Research has initiated in Fiscal Year 1971 a large-scale product-oriented and goal-oriented program. The present activities of the CDB are designed to move swiftly in the short and long-range approaches to contraceptive technology within the next few years.

A. Fundamental Research

The Contraceptive Development Branch programs in this area are mission oriented in that the aim is to further research activities which could broaden the development of new methods of fertility regulation.

Fundamental research supported by the CDB has focused in several areas which were considered promising for the development of new contraceptive methods. These areas represent both the female and male reproductive systems and, in many instances, these two are closely integrated.

The Oviduct

The oviduct or fallopian tube is a segment of the female reproductive tract with important biological functions. Among these are the transport of both spermatozoa and ova so that fertilization can take place as well as the subsequent transport of the fertilized egg to the uterus. The oviduct is a complex structure consisting of distinct anatomical regions and is composed of external and internal layers. The external muscular layer is responsible for the motility of the oviduct and the type of motility that this organ exhibits can either accelerate or decelerate the passage of the fertilized ovum toward the uterus. Under normal physiological conditions the fertilized ovum is delivered to the uterus at the most opportune time for implantation. Accelerated or retarded passage is detrimental to embryonic viability.

Studies conducted by investigators supported by the Contraceptive Development Branch have shown that, in the rabbit, tubal motility can be affected by the female sex hormones,[1/] estrogens, and progestins. Progestins appear to accelerate the transport of ova through the oviduct while estrogens accelerate or retard passage depending upon dose. Furthermore, a group of drugs known as antiestrogens likewise accelerate ovum transport.

The oviductal musculature is also controlled by neural mechanisms. Research is being supported to determine their nature and to test various drugs that are known to affect neural function. Specific drugs in this area have been shown to either increase or decrease tubal motility.[1/2/3/] It is conceivable that through the use of neural drugs and hormonal agents, tubal motility could be influenced in a desired and predictable fashion.

The inner layers of the oviduct have secretory cells which produce complex fluids that function as the supporting medium for both sperm and ova. Work being conducted by investigators supported by the Contraceptive Development Branch is aimed at elucidation of the nature of these secretions and at studying factors which can produce chemical changes in them.[4/] Alteration in the chemical nature of this fluid could influence the survival of both sperm and eggs and thus affect fertility. The practicality of this approach has not yet been ascertained but it is known that chemicals which have contraceptive effects do change the nature of oviduct fluid.[4/5/]

The cells at the ovarian end of the oviduct contain numerous microscopic projections called cilia. The beating of these cilia can influence the transport of eggs from the ovary into the oviduct. Investigators supported by the Contraceptive Development Branch are assessing the role played by the cilia in egg transport and also are studying the underlying hormonal conditions which influence the growth of these structures.[6/7/]

The proper understanding of oviductal function requires that observations be made on this organ when it is unexposed within the body. To achieve this, funds have been made available to bioengineers to develop miniature sensing devices that can be attached to the oviduct which, through an appropriate telemetry system, would transmit information concerning the motility of this organ.[1/8/] When developed, these instruments will permit the evaluation of the effects of drugs on the oviduct in the intact animal under relatively physiological conditions.

THE OVUM AND BLASTOCYST

Numerous studies are being supported by the CDB on the biology and biochemistry of the ovum (egg). Pertinent information has been obtained on the transfer of chemicals from surrounding media into the ovum.[9/10/] The possible damage that these chemicals have on the normal cell function is being investigated. The metabolic patterns of fertilized eggs are being examined under a variety of experimental conditions. As the cells divide prior to implantation new protein is being synthesized by them.[11/] We are seeking information regarding the mechanisms that regulate cell division. Prior to ovulation, the eggs are contained in the ovarian follicles. The rupture of the follicle is controlled by the action of specific hormones. However, it is still unknown what factors influence the action of these hormones, and what is the cause of follicular rupture. Investigators supported by the Contraceptive Development Branch are trying to determine whether a specific enzyme present in the ovarian follicles determines the action of the specific hormones.[12/13/] Hence, inhibition of enzyme activity could become a future method of fertility control.

Penetration of the egg by the sperm is a complicated process known as fertilization. The Contraceptive Development Branch is supporting studies in this area.[14][15] It has been known for some time that sperm penetration of the egg most probably requires enzyme action.[16][17][18] Research indicates that the egg membranes can be altered to resist sperm passage.[17] Is it then possible to produce such changes within the maternal system and prevent fertilization? This aspect is being pursued in several laboratories and only preliminary data are presently available.

THE CORPUS LUTEUM (CL)

The corpus luteum is an ovarian structure formed following the rupture of the follicle. Its primary function is to produce progesterone, a hormone necessary for the establishment and maintenance of pregnancy. Removal of the corpus luteum at early stages of gestation results in pregnancy failure.[19]

Disruption of corpus luteum (CL) function has been attempted by several investigators. The synthesis of progesterone in the CL involves a number of specific enzymes. These enzymes have been identified and specific inhibitors have been found which can block the production of progesterone.[20] Such inhibitors could be used therapeutically in several ways, such as for interruption of implantation or, of established pregnancy or, as a once-a-month pill. Injection of follicular fluid can interrupt pregnancy in rabbits,[21] which is accompanied by a marked decrease in progesterone levels. The identification of the factor contained in follicular fluid is contemplated.

The Contraceptive Development Branch is supporting several investigators in the prostaglandin area.[22-28] Discovered some years ago in seminal fluid, their structure has only recently been identified. Two specific prostaglandins have profound effects on the reproductive system. These substances have undergone clinical trials and have been found effective in the termination of both early and established pregnancy. The prostaglandin research being supported by the Contraceptive Development Branch includes both physiological studies regarding their mode of action and synthesis of compounds which will demonstrate prostaglandin-like activity or which will inhibit the action of known prostaglandins. This latter aspect will receive further attention during discussion of the drug development program of the Contraceptive Development Branch.

STUDIES ON SPERMATOZOA AND FERTILIZATION

In order to acquire fertilizing capacity, spermatozoa of most species must spend a certain period of time in the female reproductive tract. The process has been called capacitation. It has also been observed that addition of seminal fluid to such capacitated spermatozoa renders them incapable of fertilizing the ovum, a process called decapacitation.[17] Investigators are presently trying to identify the decapacitation factor and to see whether decapacitation can be brought about by chemicals and whether this can be a useful contraceptive method.[17][18]

Spermatozoa contain a trypsin-like enzyme that may be responsible for the passage of the sperm into the egg. This enzyme is contained in the anterior

section of the spermatozoon called the acrosome.[16/18/] It has been shown that inhibition of this enzyme leads to fertilization failure. A fairly specific inhibitor of this enzyme named TLCK has been found. This substance preferentially inhibits the enzyme in spermatozoa and, to a much lesser extent, the enzyme trypsin that is found in other parts of the body.[17/] Several Contraceptive Development Branch supported investigators are presently assessing the action of TLCK as a vaginal contraceptive, and are searching for other inhibitors that could be given systemically to males in order to inhibit spermatozoan fertilizing capacity.[17/18/]

IMMUNOLOGICAL STUDIES

Immunological approaches have been identified as one of the possible areas that may prove fruitful for fertility control. Several interesting and valuable observations have been made by researchers under our support.[3/29-31/] Antibodies to the trophoblast (a specialized area of the placenta) have been produced.[30/] Injection of gamma globulin (which carries this antibody) into pregnant monkeys has resulted in abortion in this species. The mothers given this material did not suffer from ill effects. Considerably more work needs to be carried out on the purification of this antibody in order to ascertain its efficacy and safety. The possibility that such an approach may be useful for pregnancy termination in the human must await further study.

Antibodies to extracts of testicular tissue have also been produced. When injected into experimental animals, this material produced disruption of testicular function.[31/] The substance is being purified so that the immunization procedure will induce temporary infertility while maintaining libido. Antibodies produced following injection of sperm homogenates have been shown to affect the ability of sperm to ascend the female reproductive tract.[29/] These spermatozoa retain their motility but appear to be incapable of passage through the cervix. Attempts will be made to produce antibodies to a purified enzyme that plays a role in the fertilization process and then to assess the antifertility effect of this procedure.

STUDIES ON HORMONE ACTION

An understanding of how a hormone exerts its effect on a target organ is of the utmost importance. Current analysis of male and female sex hormone action indicates that these substances attach themselves to specific receptors in the target tissues.

Studies supported by the Contraceptive Development Branch programs have been centered on several problems in this general area.[32-36/] For example, is the chemical nature of these receptors the same in all target tissues?[32/35/] Results to date seem to indicate that while the receptors are similar their quantity in different tissues varies.[32/] Numerous antagonists of estrogen, the female sex hormone, have been described. However, antagonists of the pregnancy maintaining hormone, progesterone, are being actively sought. Research on the characteristics of progesterone binding may suggest the structural requirements for an antiprogestin which could become a useful drug in the area of fertility regulation.[33/]

The functions of the ovary and the testes are controlled by pituitary hormones called gonadotropins. Several contracts in this area are supported with CDB funds.[37-40] The studies are designed to (1) determine the mode of action of gonadotropins on the male and female gonad, (2) precisely quantitate this action and (3) correlate fluctuations in the levels of these pituitary hormones with fluctuations in male and female sex hormones. The ability to quantitate hormone levels in body fluids is important if we are to understand the various interrelationships that result in fertility control. The breaking down and rebuilding of one of the gonadotropins called luteinizing hormone is being studied.[40] Oral contraceptives interfere with the release of this important hormone. The knowledge of the structure of this hormone may lead to its synthesis and to the synthesis of compounds which may inhibit its production or action.

B. PRODUCT-ORIENTED AND GOAL-ORIENTED CDB PROGRAMS

During FY 71 the Contraceptive Development Branch has initiated several new programs in this area: (1) drug development programs, (2) the program related to the development of techniques for permanent and reversible sterilization of both sexes, (3) a program related to the development of techniques for the disruption of early pregnancy, and (4) a program related to the development of drug delivery systems.

1. DRUG DEVELOPMENT PROGRAM

The need for new contraceptive drugs is very real. In the past few years there have been no new contraceptive drugs introduced on the market. Several new formulations involving already accepted drugs have appeared. The intent of these formulations has been to increase the safety of the combined progestin-estrogen pills. Several alternatives are open in the drug area. One involves the synthesis of new steroidal compounds which either have demonstrated unusual antifertility activity or which can be expected to demonstrate a specific hormonal activity known to be useful in the antifertility area. Developments in this area might result in a multiplicity of therapeutic regimens which could conceivably include once-a-month long-acting drugs, postcoital agents or anti-implantatory agents. Synthesis of nonsteroidal antifertility agents is also encouraged. As with some of the steroidal agents, antifertility utility and safety is yet to be documented. Support of synthesis of prostaglandin-like compounds may yield clinical utility. Drugs in this area could interfere with corpus luteum function as luteolytic agents, stimulate uterine activity, act as abortifacients or induce menses at regular monthly intervals used as a once-a-month pill. The naturally occurring prostaglandins have a number of side effects which impose a limitation on their widespread utility. Hopefully, some of the newly synthesized prostaglandins or their antagonists may prove to be useful without having the concomitant liability of undesirable side effects. Development of male contraceptives which maintain libido while at the same time inhibiting sperm production appears to be a realistic program for the not-too-distant future. Contracts in all these areas will be funded during FY 71.

2. DEVELOPMENT OF STERILIZATION TECHNIQUES

The aim of this program is to develop new techniques to accomplish reversible sterilization of both males and females. The acceptability of present day sterilization techniques is hampered by the relative permanency of the surgical sterilization techniques. A variety of procedures and devices have been proposed to achieve reversible sterilization. These projects will be funded during FY 71.

3. DISRUPTION OF EARLY PREGNANCY

Research is now in progress to evaluate new methods of abortion. One investigator, supported on CDB funds, has observed that the application of heat to the uterus in terms of microwave radiation disrupted pregnancy in rats without harming the mothers.41/ Several other methods of abortion induction have been proposed and hopefully some of these may find clinical utility.

4. DRUG DELIVERY SYSTEMS

Historically, drugs are administered in single or multiple doses. Developments within the past few years indicated that small amounts of a drug can be delivered at a relatively constant rate by introducing it into capsules made of special material and implanting these capsules under the skin or by introducing them into the vagina. Several proposals on new methods of drug delivery systems will be funded during FY 71. Some of these represent alterations in available technology and others represent new advances with improvement in this area.

C. CDB ACTIVITIES IN THE AREA OF HORMONE DISTRIBUTION

Investigators in the field of reproductive biology have found it difficult to obtain specific hormones for their studies. Even when these are available they do not always represent pure material. When this occurs duplication of results among different laboratories is very difficult. The CDB has contracted with several organizations to prepare adequate quantities of specific hormones which will then be made available to qualified investigators.42/43/ This will hopefully save time, effort and funds and reduce the disparity that exists in certain specific scientific areas.

D. PUBLICATIONS RESULTING FROM CDB ACTIVITIES

Total number of papers presented at meetings of scientific societies: twenty-nine (29). Total number of papers published: twenty-seven (27). Total number of papers submitted for publication: twenty-eight (28).

REFERENCES

1. Blandau, R. J., 70-2141, $195,890. An interdisciplinary project on the biology of the oviduct and gamete transport. University of Washington

2. Black, D. L., 69-2132, $12,200. Neural control of the mammalian oviduct. University of Massachusetts

3. Feigen, G. A., 70-2157, $40,793. Immunopharmacology of the human fallopian tube. Stanford University

4. Brunton, W. J., 70-2046, $41,362. Factors influencing mammalian oviductal secretions. Johns Hopkins University

5. Leppi, T. J., 70-2311, $17,763. Morphochemical analysis of mucins in the mammalian oviduct. University of New Mexico

6. Odor, D. Louise, 70-2142, $13,195. Ultrastructural studies on the oviduct of intact normal, the ovariectomized and ovariectomized-hormone treated rabbit and pig-tailed monkey, Macaca memestrina. Medical College of Va.

7. Brenner, R. M., 70-2146, $14,831. Control of ciliogenesis in the primate oviduct. University of Oregon

8. Fromm, E., 69-2189, $38,437. A bioengineering approach to the study of tubal activity in contraception. Drexel Institute

9. Glass, L., 70-2309, $29,000. Transfer of maternal macromolecules to mammalian eggs. University of California

10. Cross, M., 69-2141, $28,697. Blastocyst expansion and preovulatory follicle swelling. University of Pennsylvania

11. Hahn, W., 70-2259, $26,197. The effect of progesterone and estradiol on RNA synthesis in the rabbit oviduct and its relationship to early embryonic development. University of Colorado

12.. Espey, L., 69-2126, $51,493. The physiology of ovulation. Trinity College

13. Mandl, A., 70-2252, $28,300. The mechanism of follicular rupture. Columbia University

14. Brackett, B., 70-2163, $48,740. In vitro fertilization of primate ova. University of Pennsylvania

15. Cross, P., 70-2150, $39,945. Mammalian oocyte maturation and fertilization in vitro. University of Pennsylvania

16. Srivastava, P., 70-2147, $29,494. Enzymes of the sperm acrosome, occurence of the inhibitors of these enzymes in seminal plasma and the role of the enzymes and inhibitors in capacitation of sperm and in the penetration and fertilization of ova. University of Georgia

17. Williams, W. L., 69-2103, $45,351. Biochemical requirements for fertilization and development of rabbit and human ova. University of Georgia

18. Stambaugh, R., 70-2161, $28,250. Studies on acrosomal enzymes effecting penetration of the zona pellucida. University of Pennsylvania

19. Csapo, A., 69-2194, $48,718. The action of estrogens and progesterone upon uterine function in pregnancy. Washington University

20. Salhanick, H., 70-2319, $45,120. Development of in vitro screening procedures for inhibitors of progesterone synthesis. Harvard University

21. Nalbandov, A., 69-2135, $45,635. Anti-luteinizing activity of the follicular ovum. University of Illinois

22. Fried, J., 71-2117, $46,675. Synthesis of prostaglandin analogs for biological studies. University of Chicago

23. LeMaire, W. J., 71-4-044, $52,491. Effects of prostaglandins on ovarian function. University of Miami

24. Speroff, L., 71-2118, $25,470. The role of prostaglandins in ovarian physiology. Yale University

25. Kirton, K. T., 69-2208, $127,678 (2 years). Luteolysis as an approach to contraception. Upjohn Company

26. Inskeep, E. K., 69-2215, $35,537 (2 years). Relationships of endometrial prostaglandins and luteal function. West Virginia University

27. Risley, P. L., 69-2099, $34,744. New methods of fertility regulation: prostaglandins and smooth muscle responses of male sex organs. University of Oregon

28. Hansel, W., 69-2209, $107,232 (3 years). Isolation of bovine uterine luteolytic factor. Cornell University

29. Metz, C. B., 70-2253, $92,300. Immunochemical investigation of sperm transport and fertilization in the mammal. University of Miami

30. Behrman, S. J., 70-2155, $38,229. The antigenicity and immunology of the trophoblast and the potentials for the use of anti-trophoblast serum as a contraceptive. University of Michigan

31. Goldberg, E., 69-2162, $45,061. Biochemical studies on spermatogenesis and spermatozoa. Northwestern University

References con't

32. Notides, A., 70-2254, $33,450. Studies of the estrogen-binding protein in the human uterus and anterior pituitary and the rodent uterus, anterior pituitary and the hypothalamus. University of Rochester

33. Toff, D., 70-2165, $40,088. Studies on uteroid hormone receptors and their role in reproductive biology. Vanderbilt University

34. Bardin, C. W., 71-4-017, $20,694. Assay of progesterone receptor activity in the guinea pig uterus. Milton S. Hershey Medical Center

35. Eisenfeld, A. J., 70-2258, $50,795. Estrogen binding in reproductive organs. Yale University

36. Jensen, E. V., 69-2108, $29,815. Estrogen receptor substances of oviduct tissue. University of Chicago

37. Niswender, G. D., 69-2134, $37,219. Endocrine regulation of the corpus luteum. University of Michigan

38. Falb, R.D., 70-2308, $21,165. Solid phase radioimmunoassay of hormones. Battelle Memorial Institute

39. Williams, W. L., 70-2262, $33,500. Study of gonadotropin action in the testes. University of Georgia

40. Ward, D., 69-2221, $119,340 (3 years). Physiological aspect of the attachment of carbohydrate to luteinizing hormone. University of Texas

41. Brent, R. L., 70-2306, $37,660. Experimental methods of interrupting pregnancy. Jefferson Medical College

42. Shipley, E. G., 70-2250, $78,553. Radioimmunoassay of Rhesus monkey gonadotropins. Endocrine Laboratories of Madison Inc.

43. Canfield, R. E., 70-2251, $47,300. Pilot study for the large scale preparation of purified human chorionic gonadotropin. Columbia University

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Symposium on Environment and Reproductive Processes
Contractor: Ohio State University
Money Allocated: $5,000 (FY 71 Funds)

Objectives: To reach a better understanding of the problems and possibilities involving ecological situations in reproduction. To clarify how sexual reproduction is regulated by the state of an organism's internal and external milieu.

Significance to Biomedical Research and Program of the Institute: The knowledge of the interaction between the reproductive system and its enviorment is important to the goals of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Conference on Oogenesis
Contractor: Johns Hopkins University
Money Allocated: $17,414 (FY 71 Funds)

Objectives: To review in depth the processes involved in the development of mature ova. Current investigations and the present state of the art will be assessed, as well as future goals and areas for future investigation.

Significance to Biomedical Research and Program of the Institute: It is pertinent to critically examine our basic knowledge on oogenesis, in view of the programs's desire to devise new contraceptive measures.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Symposium on Immunoreproduction
Contractor: Society for the Study of Reproduction
c/o Rutgers University
Money Allocated: $4,000 (FY 71 Funds)

Objectives: To acquaint the participants of the meeting with the importance of immunology as it relates to the reproductive processes in the male and female, to provide new insights as to the biological effects of antibodies on reproductive processes and indeed on their potential for reproductive control and to examine the role of immunology in the infertile human.

Significance to Biomedical Research and Program of the Institute: Immunological phenomena play an important role in the reproductive process and are being investigated as a method of fertility control. This area has been identified as a particularly important topic for the study for contraceptive development program of the Center.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Symposium on Biology of the Cervix
Contractor: University of Washington
Money Allocated: $24,305 (FY 71 Funds)

Objectives: In light of the significant role of the cervix in fertility and infertility and the need to develop effective contraceptives it is important to assess and evaluate the present state of knowledge about the cervix; to identify areas of research where new approaches may accelerate research development; to formulate and to propose research problems that need interdisciplinary efforts for their solution; to identify and encourage investigators outside the field of medicine and biology, e.g., biophysicists, bioengineers, etc., whose talents may contribute to effective progress in solving the complex problems of basic human reproduction and family planning.

Significance to Biomedical Research and Program of the Institute: The study of the biology of the cervix has been designated as an area of priority attention by the Center for Population Research.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Fourth International Congress of Endocrinology
Contractor: Emory University
Money Allocated: $20,000 (FY 71 Funds)

Objectives: To further our knowledge in the area of endocrinology of reproduction.

Significance to Biomedical Research and Program of the Institute: The Endocrine Congress will bring together the outstanding investigators in Reproductive Endocrinology. A significant portion of the program will be devoted to various aspects of reproduction. Subjects to be discussed are of primary interest to the goals of our program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 71-2117
Contract Title: Synthesis of Prostaglandin Analogs for Biological Studies
Contractor: Ben May Laboratory for Cancer Research
Money Allocated: $46,675 (FY 71 Funds)

Objectives: The objective of this contract is to synthesize prostaglandins and prostaglandin analogs with the purpose of making available both the active materials and possible antagonists in relatively large amounts for intensive biological investigations.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on prostaglandins which have potential utility in the control of reproductive processes.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 71-2118
Contract Title: The Role of Prostaglandins in Ovarian Physiology
Contractor: Yale University
Money Allocated: $25,470 (FY 71 Funds)

Objectives: The objective of this research is to study the effects of prostaglandins on ovarian physiology in the rhesus monkey. Specifically, this entails a study of 1) the effects of prostaglandins on steroidogenesis in the developing follicle and corpus luteum; 2) the effects of prostaglandins on development and morphology of the follicle and corpus luteum, and the effect on ovulation; 3) the effects of prostaglandin antibodies and antagonists on ovarian function.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the effects of prostaglandins on ovarian physiology.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2096
Contract Title: Biochemical and Pharmacologic Studies on the Mammalian Oviduct
Contractor: University of Cincinnati College of Medicine
Money Allocated $33,500 (FY 69 Funds), $36,540 (FY 70 Funds)

Objectives: The general purpose of this project is to examine the means by which the oviduct provides the specialized environment required by the fertilized ovum in its early stages, and to evaluate the mechanisms which control tubal activity and function. Inasmuch as pyruvate lactate, and bicarbonate are vital materials for ovum sustenance, their pathways of formation in oviduct, as compared to those in liver and muscle, and the influence of hormonal environment on these pathways are being studied in detail, both in human and in guinea pig tissue. The content and identity of the prostaglandins in oviduct tissue as a function of stage in the estrous cycle is also being determined. The effects on tubal motility of steroid hormones, prostaglandins, and catecholamines, and the influence of adrenergic blocking agents on these effects are being examined. The relation of cyclic AMP to the above Fallopian tube pharmacophysiology is being investigated using a recently developed bioassay for this nucleotide.

Major Findings: The human oviduct is capable of forming prostaglandins from the appropriate fatty acid precursors. The type of prostaglandin formed by the human tube was primarily the PGA type possibly reflecting PGE formation. The PGF prostaglandin was conspicuously absent.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the metabolism and biochemical function of oviductal tissues.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2108
Contract Title: Estrogen-Receptor Substances of Oviduct Tissue
Contractor: University of Chicago
Money Allocated: $45,413 (FY 69 Funds), $29,815 (FY 70 Funds), $31,944 (FY 71 Funds)

Objectives: The investigators are studying the molecular mechanism of estrogen action in oviduct tissue by biochemical and autoradiographic determination of the presence, intracellular localization, and chemical nature of specific "estrogen receptor" proteins.

Major Findings: It has been determined that oviduct tissues of the calf and the rat contain an estrogen receptor system similar in its general characteristics to that previously demonstrated in uterine tissue. Although the general pattern of estrogen-receptor interaction is similar in uterus and oviduct, there may be certain differences in the specific receptor substances which could be responsible for unique effects of estrogen in oviduct tissue. For example, in the rat, the nuclear estradiol-receptor complex of uterus appears to sediment slightly faster than that of ampulla and isthmus. There is a difference in the effect of temperature on estradiol uptake in vitro: unlike rat and calf uterus, which show a greater uptake of estradiol at 37° than at 25°, calf oviduct tissue (especially the fimbria) does not show this temperature dependence. Finally, data indicates that the binding affinity of receptor protein in oviduct cytosol may not be as strong as in the uterus.

Significance to Biomedical Research and Program of the Institute: This particular study is an important step in understanding hormone and possibly anti-hormone action at the cellular level and is directly relevant to the purposes of the Contraceptive Development Program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2131
Contract Title: Proteins of the Oviduct and Oviductal Fluid
Contractor: Columbia University
Money Allocated: $30,708 (FY 69 Funds) $24,688 (FY 70 Funds)

Objectives: This project is concerned with determining the protein composition of oviductal tissue and fluids, in the hope that, by appropriate modification of this composition, the tubal environment can be rendered hostile to viability or transport of sperm or ova, the fertilization process, zygote viability, or blastocyst development and transport. The effect of steroidal hormones on oviductal proteins, and the enzyme carbonic anhydrase are being investigated throughly.

Major Findings: Oviductal fluid from estrogen-stimulated rabbits contains a protein that appears to be estrogen dependent and is different from serum proteins.

Significance to Biomedical Research and Program of the Institute: Studies of the proteins of oviductal fluids are particularly relevant to the purposes of the contract program.

Proposed Course: Termination

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2132
Contract Title: Neural Control of the Mammalian Oviduct
Contractor: University of Massachusetts
Money Allocated: $14,955 (FY 69 Funds), $14,840 (FY 70 Funds), $13,850 (FY 71 Funds)

Objectives: Using rabbits in which the oviducts have been denervated by stripping vaginal and recto-vaginal septal fascia and removing the inferior mesenteric ganglion, spermatozoa and ova transport are being studied both in the denervated state and in the presence of autonomic drugs. An initial stage of the program is involved with establishing the effectiveness of the denervation procedure, using the disappearance of norepinephrine from the oviduct as an index. Rabbits undergoing this procedure are mated after 10 days. In order to determine the migration rate of spermatozoa, the tubo-uterine junction of one side is ligated at 1, 2, 3 or 4 hours post coitum. Forty-eight hours later, the animals are killed and the oviduct flushed to recover ova for study by phase microscopy for evidence of fertilization.

Ovum transport is also being studied in rabbits mated 10 days after the denervation procedure. Animals are killed 24, 36, 48, 60, 72 and 84 hours post coitum and laparotomy immediately performed. The viscera are rapidly frozen to stop oviduct motility and, after thawing, the oviduct are cleared in order to determine the exact position of each ovum.

Major Findings: Data is presented to demonstrate that pharmacological agents (epinephrine, phenoxybenzamine, propanolol, and pilocarpine) administered to rabbits modify the rate of gamete transport through the oviduct. In addition to altered transport, some detrimental effects on the eggs were observed. Since these detrimental effects occur with drugs with which there is normal transport (e.g. pilocarpine), the effect may be caused by alteration in oviductal fluid composition.

The reproductive tract of the rabbit has been extremely difficult to denervate. An attempt was first made to chemically denervate the oviduct or specific areas such as the ampullar isthmic junction and uterotubal junction. Complete denervation, however, was not successful. Various nerve segments were cut but that also failed to lead to complete denervation. Apparently, there are a number of routes of autonomic innervation which are difficult to detect and cut or remove.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the role of the oviduct in gamete transport.

Contract Number: 69-2132 (Page 2)

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2188
Contract Title: Further Development and Validation of a Method of Continuously Monitoring Peristaltic Activity of the Oviduct
Contractor: Southwest Research Institute
Money Allocated: $52,378 (FY 69 Funds), $50,637 (FY 70 Funds)

Objectives: Under this contract, the investigators are developing an ultrasonic through-transmission system for obtaining information concerning oviductal motility in unrestrained animals. Specifically, they have undertaken:

1. To assemble and test an ultrasonic through-transmission system.

2. To fabricate and test, *in vitro*, oviduct transducer assemblies.

3. To do preliminary *in vivo* tests in rabbits involving surgical implantation of the transducer, recording of the signal from the transducer before and after stimulating ovulation, and verification of ovulation by laparotomy.

4. To test the feasibility of using the ultrasonic through-transmission system for detecting ovulation in free-ranging primates by verifying the ability of the system to define accurately the time of ovulation in baboons restrained in special chairs.

Major Findings: Reproducible and dependable ultrasonic and electronic instrumentation and surgical techniques for chronic implantation of ultrasonic transducers has been developed. The instrumentation is capable of monitoring rhythmic contractile activity of the oviduct and uterus without significant interference by motion artifact. Through studies of exposure to pharmacologic agents, the presence of beta adrenergic receptors was demonstrated in the rabbit oviduct isthmus. Some of the transducers have survived up to five months of chronic implantation without visible damage to the transducer or toward tissue reaction.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch, to obtain information concerning normal oviductal function in laboratory animals and humans.

Proposed Course: Termination

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2189
Contract Title: A Bioengineering Approach to the Study of Tubal Activity and Contraception
Contractor: Drexel Institute of Technology
Money Allocated: $116,456 (FY 69-Funds) $38,437 (FY 70 Funds)

Objectives: The objective of the contract is to study tubal contractile activity in the unrestrained animal with respect to the various ovarian cycle phases, and to asses the role such activity plays with regard to ovum transport. The investigator has concentrated on the development of the appropriate transducers and telemetric packages, with initial attempts at animal implantation.

Major Findings: The investigator has developed a transducer of small enough dimensions that can be attached to monkey oviducts and record their motility. A telemetry system has likewise been designed in conjunction with the implanted transducer.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2205
Contract Title: A Biochemical Investigation of the Oviduct-Uterus System in the Pseudopregnant and Normal Cycling Hamster
Contractor: Worcester Foundation for Experimental Biology
Money Allocated: $37,182 (FY 69 Funds) for two years, $9,347 (FY 71 Funds)

Objectives: The objective of this research project is to determine whether there is a regulatory relationship between oviduct and ovary by examining "certain biochemical parameters in the oviduct-uterus system throughout normal estrous cycles and pseudopregnancy, and 'determining' the effects on these parameters of various experimental manipulations." This is being done in the hamster which has certain advantages for the study of ovarian regulation. At different stages in the ovarian cycle, oviduct and/or uterus are removed and the ability of these tissues to incorporate labeled precursors into RNA, protein and lipid in vitro measured. The effect of removing the oviduct or uterus on the ovarian cycle is being studied to determine whether there are luteolytic factors arising in the oviducts, or arising in the uterus but mediated through the oviduct. Similar experiments will be done in the pseudopregnant animal.

Major Findings: The incorporation of precursors into total protein, lipids and RNA has been studied in hamster oviducts and uteri. Oviduct removal does not cause significant differences in the biochemical parameters in the remaining uteri. Hysterectomy, however, results in a reduction of protein synthesis in the oviduct on all days except day 1 of the cycle. There is an increase in lipid synthesis on all days except day 2 of the cycle.

Neither oviduct removal nor hysterectomy appears to alter the type of vaginal discharges or the length of the hamster estrous cycle.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research devoted to identifying the factors responsible for corpus luteum formation, function, and regression.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2206
Contract Title: Characterization of Cell Types in the Female Reproductive Tract
Contractor: Georgetown University School of Medicine and Dentistry
Money Allocated: $31,349 (FY 69 Funds), $36,135 (FY 70 Funds)

Objectives: The primary objective of this research is the development of methods for isolating and separating epithelial cell types, Subsequently, studies of cell extracts by disc electrophoresis, and establishment of antigenic profiles of each cell type will be carried out. Preliminary work suggests that good suspensions of oviduct cells can be obtained, and that further separation by means of countercurrent streaming centrifugation will provide pure cultures of individual cell types for studies of their metabolic and secretory characteristics.

Major Findings: The investigator has successfully separated the ciliated from nonciliated cells from oviducts of the rat, guinea pig and the rabbit. It appears that cells from different regions of the oviduct have different centrifugal properties. Disc electrophoresis patterns of oviductal proteins indicate that there are 2-3 protein bands which cannot be detected in the liver, kidney, heart or serum.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2046
Contract Title: Factors Influencing Mammalian Oviductal Secretions
Contractor: Johns Hopkins University
Money Allocated: $32,090 (FY 69 Funds), $41,362 (FY 70 Funds), $42,686 (FY 71 Funds)

Objectives: The objective of this research project is to continue the study of the electrical potential difference exhibited across the serosal and mucosal surface of the rabbit oviduct as related to net ion transport. Preliminarily, they have found a net chloride transport across the membrane under short-circuited conditions. Since the net choloride flux is equivalent to the short-circuited current, it has been reasoned that the electrical phenomena can be accounted for by the active transport of chloride. They are now developing an in vitro preparation for the primate oviduct similar to that described for the rabbit, in order to extend their studies of the active transport of ions using the short-circuit technique to this species. Lastly, they wish to determine the effects of drugs and hormones on active transport in the in vitro oviduct system using this as a screening technique for potential contraceptive drugs.

Major Findings: Utilization of double isotope technique has shown that in the rabbit oviduct there is no net flux of Na. Utilizing radioactive Cl and Br he has shown that bidirectional fluxes result in a net halide flux that was equivalent to the short-circuit current. Drugs can produce changes in the electrical properties of the oviduct and among the ones that increase electrical properties are: isoproterenol , ephinephrine , norepinephrine , phenylephrine. Propanolol, a β -adrenergic blocker, could block responses to epinephrine, but phentolamine, an α -blocker, was without effect. The investigator hypothesizes that the β-stimulation is accomplished by increased synthesis of cyclic 3'5' AMP.

Prostaglandins E, and $F_{1\alpha}$ added to either serosal or mucosal baths caused an immediate rise in transmembrane potential and short-circuit current. For PGE, a dose-response relationship was established. Since the presence of copper in IUD's increases their effectiveness, analysis of the effect of $CuSO_4$ and of copper wires, added to the mucosal bath, was done. Both factors increased the short-circuit current and transmembrane potential, however, these could not be accounted by a change in net chloride flux.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the mechanisms responsible for the secretion of oviductal fluids and the influence of these fluids on gamete transport and survival.

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2046 (page 2)
Contract Title: Factors Influencing Mammalian Oviductal Secretions

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2141
Contract Title: An Interdisciplinary Program Project on the Biology of the Oviduct and Gamete Transport
Contractor: University of Washington School of Medicine
Money Allocated: $192,490 (FY 70 Funds), $177,734 (FY 71 Funds)

Objectives: This is a comprehensive interdisciplinary program project on the basic biology, physiology, and endocrinology, of oviductal function in rabbits and monkeys. Seven different specific aims are enumerated: 1) The development of techniques for direct observation of the normal function of the various segments of the oviduct in situ; 2) development of miniature sensing devices and telemetry equipment that will permit study of the normal activity of the oviduct in unanesthetized, unrestrained, rabbits and monkeys; 3) study of the electrophysiological characteristics of oviductal musculature; 4) investigation of intrinsic innervation, pharmacological responses, and the possible role of pacemakers in oviduct function; 5) Ultrastructural studies of the epithelium and musculature under different physiological conditions; 6) development of techniques of in vitro cultivation of oviductal tissue as a potential bioassay system; and 7) studies of the mechanism of sperm transport through the oviducts.

Major Findings: The rates of egg transport through the ampullae have been determined under both physiological and experimental conditions. The major conclusion that has been reached for the rabbit was that increased rate of egg transport is not seen under conditions of estrogen domination, but under conditions of estrogen withdrawl. Administration of a progestin or antiestrogen facilitated ovum transport. Work with the monkey is much less extensive. Transport of eggs from the fimbria to the ampullae has been observed. Thickness of oviduct walls precluded observation of transport through the ampulla. Oviductal activity in the monkey is lower than in the rabbit. Attaching of sensing devices may be easier in the monkey because of greater oviductal size. Development of sensing devices is progressing slowly, but the investigators are aware of the difficulties and are proceeding accordingly.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the various functions of the oviduct which are responsible for gamete transport and survival, fertilization, and transport of the fertilized egg to the uterus.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2142
Contract Title: Ultrastructural Studies on the Oviduct of the Intact Normal, the Ovariectomized and Ovariectomized-Hormone Treated Rabbit and Pig-Tailed Monkey, Macaca-Nemestrina
Contractor: Medical College of Virginia
Money Allocated: $13,195 (FY 70 Funds) $12,764 (FY 71 Funds)

Objectives: This research project is part of an interdisciplinary research program on oviduct structure and function being carried out at the University of Washington. This investigator will carry out at the University of Virginia certain morphological aspects of the work on specimens obtained in Seattle where correlative in vivo observations and physiological measurements are being made on the same material.

A systematic study of the ultrastructure of the fimbria, ampullae, and isthmus of the normal rabbit oviduct in the pre-and post-ovulatory period, in the ovariectomized animal, and in ovariectomized animals receiving estrogens and progesterone singly and in combination will be made. Studies will not be confined to the epithelium but will include fine structural observations on the smooth muscle which may be correlated with changes in oviduct motility. With this base line established on rabbits, similar studies will be made on the oviduct of the pig-tailed macaque.

Major Findings: Attention has been directed primarily to the examination of the fimbrial epithelium because it appears that cilia in this region play a role in the movement of ovulated eggs from the ovary to the ostium of the oviduct. Long term ovariectomy reduces the number of ciliated cells. The height of the cilia in these cells is lower than in estrous animals. No secretory activity was evident in the secretory cells. Estrogen administration to ovariectomized rabbits can either decrease or increase the proportion of ciliated cells. The nature of the response is dependent on (1) length of ovariectomy period: (2) dose of estrogen, and (3) duration of estrogen treatment. In animals ovariectomized for 28 weeks extensive estrogen treatment produced further deciliation.

In the pig-tail monkey at mid-cycle the fimbria and ampullae have approximately the same number of ciliated cells (52 and 51% respectively).

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the ultrastructure of the mammalian oviduct and its responses to changes in the hormonal environment.

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2142 (page 2)
Contract Title: Ultrastructural Studies on the Oviduct of the Intact Normal, the Ovariectomized and Ovariectomized-Hormone Treated Rabbit and Pig-Tailed Monkey, Macaca-Nemestrina

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2144
Contract Title: Measurement of Blood Flow in the Mammalian Oviduct
Contractor: Washington State University
Money Allocated: $14,065 (FY 70-Funds) $14,220 (FY 71 Funds)

Objectives: This research project is designed to measure blood flow to the mammalian oviduct and to describe the structural geometry of the oviductal vasculature. For the latter objective, silicone rubber elastomer will be injected, and casts prepared in the Anatomy Department and studied grossly with the dissecting microscope, and microscopically, with 100 to 150 micron sections. For measurement of blood flow volume, two methods are proposed: (1) indicator-diffusion using hydrogen gas; and (2) thermoconductivity. In the first method, blood flow is related to the rate of removal of hydrogen from the tissue, while in the second, the rate of dissipation of heat is the variable related to flow. These methods have been selected because they offer the best opportunity to estimate capillary flow, and appear to be adaptable for use in unanesthetized animals. Two transducer configurations will be tested: (1) a needle type, with the measuring element inserted into the tissue; and (2) a cuff type in which the measuring device is in contact with the surface of the oviduct. During acute experiments the 4-amino-antipyrene indicator-diffusion technique will be utilized for comparison purposes in the measurement of uterine blood flow. This technique will be modified using radioiodinated antipyrene when the oviduct comparison is made. Eventually, it is planned to study the effects of sex hormones on oviductal blood flow in the oophorectomized animal.

Major Findings: 1) the investigator has injected a number of rabbits with silicone rubber material and has obtained a cast of the oviduct vasculature. The primary supply of blood comes from the ovarian artery with a limited contribution from the utero-ovarian artery. 2) Electrode work has been centered on shape and composition of material and it appears that flat electrodes made of platinum are most promising for the hydrogen desaturation method. 3) In in vitro studies the relationship between hydrogen desaturation and flow rate has been established. Blood flow has been measured in acute experiments in the rabbit using the above method. Values reported are not very extensive, but do appear to be uniform. The flow rates reported are constant with values reported by other investigators for uterine blood in unanesthetized women.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies on the vascular supply and blood flow patterns in the mammalian oviduct.

Contract Number: 70-2144 (page 2)

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2146
Contract Title: Control of Ciliogenesis and Secretory Cell Growth in the Primate Oviduct
Contractor: Oregon Regional Primate Research Center
Money Allocated: $14,831 (FY 70 Funds),$11,390 (FY 71 Funds)

Objectives: The primary objective of this research project is to use this system for fundamental studies of how hormones regulate cell differentiation in the oviduct. Autoradiographic studies on amino acid uptake and protein synthesis will be carried out with in vivo and in vitro administration of labeled precursors. It is hoped thus to determine sites of synthesis of microtubal protein and the mode of growth of ciliary shafts. By use of tritiated thymidine and tritiated uridine applied at critical periods in the process of ciliogenesis, the investigator hopes to shed light on the controversial question of the presence of DNA in basal bodies and of RNA in basal body precursors.

Major Findings: (A) Attempts to label basal bodies with ^{3}H-thymidine have been partially sucessful. Initial studies, however, have shown that DNAse did not diminish the radioactivity of the basal bodies. This was most probably due to the fixative used. Changing the fixative may improve the situation. Rather than using radioactive thymidine, radioactive actinomycin D is presently being used. In initial studies employing EM autoradiography it was shown the DNAse lowered the count of grains in the basal bodies, whereas RNAse was without effect. Increasing the exposure time of the autoradiographs from one to two months doubles the grain counts.
(B) Analysis of RNA synthesis utilizing tritiated uridine indicate that RNA synthesis in differentiating cells is completed by day 3, of the estrogen injection schedule. Light microscopy shows that 6 days after exposure to uridine there is a substantial amount of label in the nucleus of the ciliated cells. Most of the label in the secretory cells is in the cytoplasm. EM autoradiograms have indicated that pulse labeling with H^{3}uridine does not label the fibrous granule precursor, indicating no RNA synthesis. These studies also revealed that the resolution of the technique is not great enough to permit one to distinguish between labeled basal body precursors and labeled ribosomes or other cytoplasmic RNA.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the formation and hormonal dependence of oviductal cilia and the role these cilia play in gamete transport.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2146
Contract Title: Control of Ciliogenesis and Secretory Cell Growth in the Primate Oviduct
Contractor: Oregon Regional Primate Research Center

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2148
Contract Title: Bioelectric Activity of the Oviduct In Vivo.
Contractor: University of Missouri
Money Allocated: $31,372 (FY 70 Funds)

Objectives: This research is aimed at the study of bioelectric activity of the oviduct in vivo, including studies of pressure changes, contractile activity, and temperature measurements, with a view to the increased comprehension of the physiological function of the oviduct in ovum transport. Particular attention will be paid to assessment of the existence of pacemakers in the oviducts. In order to carry out these studies, appropriately small transducers, electrodes, and telemeters will be developed. Initial tests of these transducers will be carried out by direct wire from the transducer implanted in situ, to recording equipment nearby. When a successful transducer configuration has been developed, in vivo studies will be attempted utilizing telemetry packages contained in back packs on goats. Only after both the transducer and telemetry packages have been shown to be effective in obtaining and sending reliable data from unrestrained animals will totally implanted systems be attempted. Once developed, the systems will be used to characterize electrical and mechanical activity of the oviduct and to relate this to uterine bioelectric activity.

Major Findings: The bioelectrical sensors and assemblies that were developed were implanted on the oviducts of two goats, one nonpregnant and one pregnant. The animals recovered from surgery rapidly and recording of bioelectrical signals was undertaken. In the nonpregnant goat bursts of rather simple pulse-like signals of about 100 microvolts were observed. There appears to be a definite positive relationship between bioelectrical activity of the oviduct and estrus. In the pregnant animal repetitive complex signals of about 400 microvolts were observed. Their rate of appearance is lower than is the estrus animal and is followed by extended periods of quiescence. The investigators feel that the probability is quite high that these recordings represent signals from the oviducts.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of appropriate instrumentation for obtaining information concerning normal oviduct activity in the unrestrained animal.

Proposed Course: Termination

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2153
Contract Title: Electron Spin Resonance and Radioisotope Labelling Studies of Mammalian Reproductive Cell Membranes in the Evaluation of New Methods of Contraception
Contractor: University of Hawaii School of Medicine
Money Allocated: $56,099 (FY 70 Funds), $66,606 (FY 71 Funds)

Objectives: In this research project, electron spin resonance (ESR) spin-labelling, will be applied to the study of sperm membrane interactions and sperm transport in vivo. The method relies on the ease of labelling sperm cells with a lipid-seeking spin-label which causes no loss of sperm motility or function. The spin-label is capable, through analysis, of reflecting structural changes in the membrane of the sperm. These changes will be measured during sperm capacitation and interaction of sperm with genital fluids and chemical contraceptives. The same kind of label, but tagged with C^{14}, will be used to measure in vivo transport of sperm in the uterine tract of experimental animals especially primates.

Following preliminary work aimed at establishing the feasibility of using such a spin-label for detecting alterations in sperm membrane characteristics, such studies would be extended to the in vivo case. Miniaturized electron spin resonance detectors would be developed for implantation directly in the tract so that alterations could be monitored directly. The investigators also propose to use a radioisotope label for studying sperm transport within the female tract. In this case the sperm would be labelled with a fatty acid carrying not a paramagnetic nitroxide, but a heavy concentration of C^{14}; the investigators have designed a small Geiger-Mueller tube (2mm diameter, 5 mm in length) which could be implanted in the uterine tract to detect in vivo transport of the labelled sperm.

Major Findings: The investigators were successful in spin labeling rabbit and hamster spermatozoa. They found that combination of nitroxide with derivatives of cholesterol or stearate gave them stable labels that did not affect sperm motility. They have been successful in attaching sufficient label to sperm to be able to detect 10^3 sperm in the oviduct. They have shown that changes in sperm membranes result in changes in the ESR spectrum. Addition of barbituric acid in sucrose to spin-labelled rabbit sperm shows that as the acrosome is disrupted and removed, the ESR spectrum of the spin label changes from a partially immoblized state to a completely immoblized state.

In their studies on the effect of capacitation on ESR they have demonstrated that in vivo capacitation of the rabbit sperm does alter the spin label. Some differences in spectra are observed in uterine sperm as compared to oviductal sperm. With hamster sperm capacitated in vitro they have shown that

Contract Number: 70-2153 (page 2)
Contract Title: Electron Spin Resonance and Radioisotope Labelling Studies of Mammalian Reproductive Cell Membranes in the Evaluation of New Methods of Contraception

there exists an intimate relationship between the capacitation protein and the sperm membrane which results in a tightening of the lipid environment of the spin label.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies which make use of state-of-the-art technology to study reproductive processes such as sperm capacitation and transport.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2157
Contract Title: Physiology of the Oviduct: Studies of Permeability and Transport Characteristics of Oviductal Epithelium; Characterization and Quantitation of Tubal Anaphylaxis
Contractor: Stanford University
Money Allocated: $40,793 (FY 70 Funds), $79,881 (FY 71 Funds)

Objectives: The work to be carried out under this contract involves two aspects of oviductal physiology: (1) characterization of the permeability and transport characteristics of oviductal epithelium; and (2) characterization and quantitation of the oviductal response to the anaphylactic reaction between an antigen and the specific antibody bound to oviductal cells. Work will be carried out in laboratory animal and human oviductal tissue, in vitro.

Major Findings: The investigator has titrated the activity of the isolated human ampulla and isthmus to histamine, serotonin, acetylacholine, epinephrine and norepinephrine. Based on contractions, the isthmus was found to be more sensitive to these drugs than the ampulla. A system has been devised to demonstrate anaphylaxis in vitro employing slices from the ampullapy and isthmic regions of the oviduct. Release of histamine was the end point. Data indicate that the isthmus had a greater histamine content than the ampulla and that it released more histamine during anaphylaxis than the latter region. The ampulla was shown to have greater histaminase activity than the isthmus and was more sensitive to histaminase inhibitors.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support innovative studies on the physiological and pharmacological properties of laboratory animal and human oviductal tissue.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:	70-2159
Contract Title:	Effects of Steroid Hormones on RNA and Protein Synthesis in the Mammalian Oviduct
Contractor:	The University of Texas at Austin
Money Allocated	$40,459 (FY 70 Funds) $21,700 (FY 71 Funds)

Objectives: The objective of this research project is to study RNA and protein synthesis in the mouse, rabbit, and guinea pig oviduct as a function of stage in the estrous cycle, time after hormone treatment, or sequence of hormone treatment in the ovariectomized animal. If initial findings indicate that there is evidence of hormonal control of genetic transcription and translation in the oviduct, the investigators will proceed with a more precise type of documentation of such hormonal control, including studies of the various anatomical regions of the oviduct. In vitro studies of whole nuclei and of template activity in vitro of isolated and purified chromatin with added RNA polymerase are also planned. It is hoped to be able to isolate and characterize oviductal polyribosomes and to analyze their amino acid incorporation activity in vitro. All these responses will be measured after different hormone treatment in spayed animals or at different stages of the estrous cycle in intact animals. Eventually, the investigators plan to seek further information by techniques of RNA-DNA hybridization and competition experiments.

Major Findings: The data indicates that in the mouse oviduct the content of protein, RNA are highest during proestrus. The amount of DNA does not tend to fluctuate to any great extent during the cycle. Both RNA and protein content of the oviduct are primarily under estrogenic control and progesterone may exert a suppressive effect. Unlike the situation in the uterus, progesterone did not antagonize the increase in oviductal weight produced by estradiol. Mating has little effect on oviductal biochemistry during blastocyst transport. Part of the work described above has been accepted for publication.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the synthetic capabilities of the mammalian oviduct and their influence by hormonal environment.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2164
Contract Title: Control of Oviduct Functions
Contractor: Clemson University
Money Allocated: $65,408 (FY 70 Funds)

Objectives: The objective of the research project is to develop the methodology for studying, in the unrestrained sheep, the integrated activity of the oviduct and its modulation and control by endogenous hormonal conditions. The specific objectives are: 1) to develop and test a transducer-telemetry system for the measurement of oviductal contractions; 2) to develop suitable programs for the data reduction and correlations necessary to provide a real-time analysis method for oviductal contractile activity; 3) to relate oviductal mechanical activity to endogenous environment (LH, estrogen, and progesterone); 4) to study the integrated activity of the oviduct as it concerns fertilization and embryo development; and 5) to determine the effects of hormones, drugs, etc., on oviduct activity, fertilization and embryonic development.

Major Findings: This study has not advanced beyond the fabrication phase of several transducer models and major findings are not available at this early stage of development.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of techniques for studying oviductal function in vivo.

Proposed Course: Terminated

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2165
Contract Title: Studies on Steroid Hormone Receptors and Their Role in Reproductive Biology
Contractor: Vanderbilt University
Money Allocated: $40,088 (FY 70 Funds), $47,941 (FY 71 Funds)

Objectives: The objectives of this research project are: (1) to study further the already established interaction of estrogens with receptor substances of rat uterus in an effort to elucidate the role of the receptors in hormone action; and (2) to investigate estrogen and progesterone localization and binding in chick oviduct, a tissue which recently has been reported to contain a specific binding protein for progesterone.

Major Findings: The investigator has attempted to study the effect of various intracellular components on sedimentation properties of the uterine estrogen receptor proteins. It has been observed that divalent cations such as Mg^{++}, Mn^{++}, Zn^{++} and Ca^{++} produced aggregation of receptor protein. Spermidine, which is an important biological polyamine likewise produced aggregation. Polynucleotides such as DNA, polyguanine and polyuridine also change the sedimentation of the cytoplasmic estrogen-receptor protein. With regard to the progesterone-binding proteins, they were found in both the nuclear and cytoplasmic fraction of chick oviduct. The binding first appears in the cytoplasm and in this form it is transported to the nucleus. To further study the hormone-protein interaction they have developed two binding assays that speed up the measurement of hormone-protein interaction. The two assays are (1) the glass absorption assay and (2) dextran-charcoal absorption assay. The utilization of these two assays has enabled the investigator to conduct competitive binding assay with a variety of steroids. Competitive binding was evident for progestins and closely related compounds. It appears that these studies may be useful in directing synthesis of highly competitive analogs.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the steroid binding substances in reproductive tract tissues.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2257
Contract Title: Physiology of the Oviduct
Contractor: Stanford University Medical School
Money Allocated: $91,585 (FY 70 Funds), $10,000 (FY 71 Funds)

Objectives: The objective of this investigation is to develop electrodes, transducers and other instrumentation for study of electrical, mechanical, pressure, and flow phenomena and to use these instruments to investigate the functional properties of the muscle coat of the oviduct (1) as small segments in the tissue bath, (2) an isolated organs and (3) in the intact subject. By determining changes in properties of the oviductal muscles as a function of hormonal environment, knowledge will be sought as to the mechanisms which control oviduct function.

These studies will involve measurements of the electrical and contractile behavior of oviductal tissue using excised oviducts of rabbit and man, at various stages of the reproductive cycle. Studies of pacemaker activity, the interrelationship of electrical activity from one area to another, correlation of electrical and mechanical activities, and intraluminal pressure changes will be conducted. The final phases of the study will involve the measurement of these functions in intact, free-roaming animals, and hopefully, eventually in women. All data will be programmed for computer analysis and a mathematical model of the system will be undertaken similar to one the investigator has developed for intestinal smooth muscle.

Major Findings: The developmental nature of this project precludes major findings at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to encourage scientists from other disciplines to apply their unique skills to studies of reproductive function.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2259
Contract Title: The Effect of Progesterone and Estradiol on RNA Synthesis in the Rabbit Oviduct and its Relationship to Early Embryonic Development
Contractor: University of Colorado Medical Center
Money Allocated: $26,197 (FY 70 Funds), $26,197 (FY 71 Funds)

Objectives: The objective of this investigation is to study RNA synthesis as a function of hormonal environment in both the embryo and the oviduct during the early stages of environment in the rabbit. Embryos, produced following superovulation, will be transferred to host females whose hormonal status will be altered by endocrine ablation, and estradiol and progesterone administration. The effects of these hormones on implantation, uridine incorporation into RNA, and the type of RNA synthesized will be determined in both the embryo and the oviduct.

Major Findings: Specifically the investigators have determined that: 1) embryos which develop in ovariectomized and sham operated animals undergo a marked increase in RNA synthesis similar to the "burst" that is seen during normal development at 60 hours post-fertilization; 2) from the *in vitro* experiments it has been tentatively concluded that continuous residence in the hormonally-supported oviduct is *not* essential for the embryo for either the 60-hour burst or the capacity for synthesizing significant amounts of ribosomal RNA; 3) employing autoradiography they have demonstrated (a) that rapid labelling occurs in the cytoplasm (28%), (b) the synthesis of the new RNA is most likely nucleoic in origin, (c) actinomycin D when introduced into the incubation medium produced marked reduction in labelling; (d) in the early rabbit embryo there do not appear to be regional differences in the rate of either RNA or protein synthesis; 4) there appear to be some differences in the fine structure of embryos recovered from ovariectomy and the sham-operated controls. The underlying cause of these differences is not known.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the hormonal control of intra-cellular processes.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2261
Contract Title: Oviduct Cilia: Structure, Function, Development
Contractor: University of California
Money Allocated: $34,343 (FY 70 Funds)

Objectives: The stroke form and fine structure of oviductal cilia is to be explored using techniques previously applied to mussel gill cilia. The aim is to provide information on the beat mechanism of oviductal cilia and to evaluate the influence of various agents on the mechanism. Techniques to be employed in these studies include high-speed cinematography, electron microscopy, and serial-section analysis of stroke form and top structure. An attempt will be made to integrate this information into a unified theory of ciliary beat based on the interaction of various ciliary organelles.

Major Findings: The ultrastructure of mouse oviductal cilia conforms to the general 9:2 pattern. Below the basal plate there is a special area called the "ciliary necklace". The "necklace" is a good candidate for the engery transducing system necessary for cilial beat. The fimbriated end of the oviduct appears as a thick carpet solidly encrusted with cilia. In the lower portion of the oviduct cilia are distinct and separate clumps arise in crevice-like protrusions.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the mechanism of action of oviductal cilia and their role in gamete transport.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2306
Contract Title: Experimental Methods of Interrupting Pregnancy
Contractor: Jefferson Medical College
Money Allocated: $37,660 (FY 70 Funds), $44,220 (FY 71 Funds)

Objectives: The investigator will investigate, in rats, two non-surgical methods for inducing abortion. The first involves microwave electromagnetic irradiation of the pregnant uterus to elevate the temperature of the fetus; the second involves the combined use of microwave electromagnetic irradiation and certain cancer chemotherapeutic agents to eliminate the fetus, without injury to the mother.

Major Findings: Utilizing conventional microwave ovens as the generating source, the investigator has modified the equipment for the use in this project. A remote control unit for monitoring, initiating, stopping, timing and varying microwave radiation was built. The antennae for directing the radiation are ready for use.

In an initial biological experiment, 20 8-day pregnant rats were laparotomized and one pregnant horn was exposed to a flux of microwave radiation which raised the temperature to 45°C for 5 minutes. The rest of the animal was shielded with aluminum foil. At term the mothers were sacrificed and the uterine contents examined. In the exposed horns only resorption sites were encountered. The fetuses in control horns were completely normal as were the mothers.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on methods of interrupting pregnancy.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2311
Contract Title: Morphochemical Analysis of Mucins in the Mammalian Oviduct
Contractor: University of New Mexico School of Medicine
Money Allocated: $17,763 (FY 70 Funds), $19,395 (FY 71 Funds)

Objectives: The investigator plans to study the mucosubstances of each of the morphologically distinct regions of the oviduct from cycling and ovariectomized hormonally treated rabbits and from humans during the menstrual cycle by a battery of chemical staining techniques generally known to be specific for the reactive groups in the carbohydrate moieties of the epithelial secretions. The effects of specific chemical and enzymatic alterations of staining patterns will be analyzed to determine the reliability of observed staining alone. The ester sulfate, carboxylate and vicinal hydroxyl groups of the mucosubstances will be stressed in an attempt to correlate their presence or absence with various endocrine states. Various radioactive precursor sugars of epithelial mucins will be used to follow the incorporation of these radioisotopic labels into the oviductal mucins. This will be done under various hormonal states.

Major Findings: The oviductal mucins have been investigated utilizing specific histo-and cytochemical techniques at the light microscope level of resolution. Of prime importance was the observation that there are distinct regional histochemical differences in the precoital rabbit oviduct. Weakly acidic mucins were present in the infundibulum. They were localized on the surface of the secretory cells. Ampullary mucins contain highly acidic carbohydrates and sulfated mucins. The isthmian portion of the oviduct contains apical and surface mucins that possess both ester sulfate and sialic acid in their carbohydrate portions. The investigators are presently conducting electron microscopic histochemical studies on the various sections of the oviduct and comparing them with the light microscope studies. There appears to be a good correlation between the two sets of observations. The ampullary and isthmian portions of the precoital oviduct have surfaces composed of highly charged (polyanionic) carbohydrate-rich materials, whereas the infundibulum is devoid of such mucin.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the oviduct.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Assay of Progesterone Receptor Activity in the Guinea Pig Uterus
Contractor: Milton S. Hershey Medical Center
Money Allocated: $20,694 (FY 71 Funds)

Objectives: The biologic action of progesterone on the uterus may be initiated by the interaction of this steroid with a specific intracellular receptor protein. In the uterus of the castrate estrogen treated guinea pig specific cytoplasmic receptors for progestins have been identified. The purpose of this study will be to characterize these receptors in order to determine the best technique for measuring their binding capacities. A rapid assay technique will be developed for uterine cytosol protein binding activity. This assay will then be used to search for agents which both block progesterone binding to the cytosol receptor and which have relatively little progestational activity. From experience with other steroid receptor systems, it is anticipated that these agents will be antiprogestational and may therefore be useful as contraceptive agents.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies on the binding characteristics of steroid receptor systems.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2133
Contract Title: The Study of Neural Regulation of Ovarian Function
Contractor: Southwest Foundation for Research and Education
Money Allocated: $29,000 (FY 69 Funds), $31,146 (FY 70 Funds), $31,146 (FY 71 Funds)

Objectives: The objective of this research is to complete stereotaxic coordinates of the hypothalamus and limbic areas of the baboon. Since external coordinates cannot be used due to excessive variation in skull shape from animal to animal, localization of the nuclei in the hypothalamus is being attempted using internal reference points obtained by positive contrast ventriculography. The animal heads are obtained at no cost from other studies.

Major Findings: Stereotaxic coordinates of the hypothalamus and limbic area in the baboon have been investigated using (1) internal reference points located by positive contrast ventriculography; (2) electrolytic lesions at given intervals both horizontally and vertically with positive reference to the ventricular landmarks for stereotaxic mapping and (3) verification of electrode placement by serial sectioning and appropriate staining. Satisfactory information has now been obtained to allow stereotaxic surgery of the hypothalamus to be carried out with good accuracy.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2134
Contract Title: Endocrine Regulation of the Corpus Luteum
Contractor: The University of Michigan
Ann Arbor, Michigan
Money Allocated: $26,706 (FY 69 Funds) $37,220 (FY 70 Funds)
$53,618 (FY 71 Funds) for 03 year

Objectives: The primary objective of this research project is to elucidate the endocrine regulation of the ovine estrous cycle by measuring peripheral serum levels of FSH, LH, prolactin, estradiol, and progesterone. Radioimmunoassay methods are being developed for all these measurements. For the steroids, the methods are validated by comparison with double isotope derivative methods. Methods are also being developed to measure uptake of progesterone-C^{14} and LH-I^{125} by uterus and ovaries respectively. Preimplantation embryos will be studied to determine if they have the ability to synthesize LH in vitro, and to metabolize sex steroids in vitro.

Major Findings: The data indicates that an increased level of LH and/or FSH and/or prolactin in serum does not appear to be the signal for maintenance of corpus luteum of gestation in sheep. In addition, regression of the corpus luteum in the cyclic animal is not due to a significant decline in serum levels of LH, prolactin, or FSH between day 9 and the next estrus. Studies on the uptake and/or metabolism of steroid and gonadotrophic hormones indicate that prolactin is bound to a component of the corpus luteum (but not endometrium follicles, ovarian stroma or psoas muscle).

Results of studies on the production of a luteotrophic substance by the sheep embryo suggest that these embryos could stimulate synthesis of progesterone. However, the investigator has been unable to demonstrate any stimulation of progesterone synthesis with serum obtained from pregnant ewes or any inhibition of progesterone synthesis with serum from the cyclic animals.

During the period of support by this contract the investigator has completed the development of radioimmunoassays for FSH and prolactin in sheep and has recently completed the development of a radioimmunoassay for progesterone.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to describe quantitatively the hormonal components of the estrous cycle in relevant animal models.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2135
Contract Title: Anti-luteinizing Activity of Follicular Ovum
Contractor: University of Illinois
Money Allocated: $43,365 (FY 69 Funds), $45,635 (FY 70 Funds), $46,941 (FY 71 Funds) for 03 year

Objectives: Recent work from this laboratory has shown that removal of the egg and surrounding granulosa cells from a ripe ovarian follicle (rabbit) initiates luteinization and a short spurt in progesterone secretion. Hence, the egg seems to exert an anti-luteinizing activity. A series of well-planned studies to elaborate and determine the significance of this observation are being carried out under the terms of this contract.

Major Findings: A single injection of LH (5ug) into the ovectomized follicle transformed it into a permanent corpus luteum which persisted for at least the duration of normal pseudopregnancy.

Large quantities of pig follicular fluid were collected and injected into rabbits immediately after mating. The abortifacient effect of follicular fluid apparently lies in its ability to reduce progesterone production. There was a statistically highly significant difference in progesterone levels in the ovarian plasma of the treated animals (174 ng/ml) compared to control animals (769 ng/ml).

Significance to Biomedical Research and Program of the Institute: Reproductive function in the human appears to depend on corpus luteum luteinization and luteolysis which have thus been identified as particularly important topics to the purposes of the contraceptive development program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2136
Contract Title: Ultrastructural Studies of Estrogen Effects on Uterus, Oviduct, Ovary, Placenta, and Blood
Contractor: University of Minnesota
Money Allocated: $18,646 (FY 69 Funds), $20,865 (FY 70 Funds), $23,357 (FY 71 Funds)

Objectives: This is a wide-ranging study of the reproductive tract by ultrastructural and radioautographic methods. The fine structure of the corpus luteum of the white-tailed deer and raccoon is being studied, and several species of animals (deer, raccoon, and green monkey) are being injected with estrogen during the anestrous and estrous periods, and samples taken at all levels of the female reproductive tract. These studies will relate ultrastructural cytology with biochemical functions, with particular emphasis on the evolutionary significance of hormonal changes.

Major Findings: Analysis of blood plasma proteins of the green monkey indicate that the observed pattern of changes in this species in response to contraceptive agents is very similar to the observations in the human.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractural effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2165
Contract Title: Corpus Luteum Regulatory Mechanisms
Contractor: University of Western Ontario
London, Ontario, Canada
Money Allocated: $36,508 (FY 69 Funds) $45,208 (FY 70 Funds)
$47,232 (FY 71 Funds) for 03 year

Objectives: This is a study of the luteinization process of the granulosa cell at the time of ovulation, as well as of the process of luteolysis. The investigator previously has shown that autotransplantation of follicles to beneath the kidney capsule enables the process of luteinization to be studied independently of changes which may occur in other ovarian components. Using this approach he is investigating the role of LH in luteinization. If LH produces luteinization, possible intermediates in the action of LH such as cyclic AMP, RNA, and protein fractions from LH-treated follicles can then be substituted. To test the hypothesis that luteinization of granulosa cells may be secondary to increased permeability of the basement membrane, follicles are pre-incubated in diluted solutions of hyaluronidase. Finally, the influence of steroids such as progesterone, dihydroprogesterone, estrogens, etc., is being evaluated by the addition of the steroids to the follicles during a pre-incubation.

A study of luteolytic factors is also being conducted in the rat, guinea pig and rabbit in a search for a species which models the human in its biosynthesis of steroids and its steroid phospholipid and intracellular localizations. LH, LTH, estrogen, progestins and hypophysectomy are being examined for their effects on corpus luteum function and regression.

The human corpus luteum will be studied by incubation of corpora lutea removed surgically in normal cycles and after the administration of estrogens and progestins. Plasma LH and progesterone will also be determined. The luteotrophic effects of estrogen will be assessed as well as estrogen inhibitors.

Major Findings: Investigations of factors responsible for initiation of luteinization of rat ovarian follicles have been carried out using a technique of *in vitro* incubation of follicles, followed by homo-or auto transplantation beneath the kidney capsules in hypophysectomized rats. Results indicate a specific action of LH in initiating luteinization and suggest that basal levels of LH secretion in intact rats are sufficient to induce lutenization. Dibutyryl Cyclic AMP also initiates luteinization. Progesterone, on the other hand, neither induces luteinization when added to incubation media in absence of LH nor inhibits the response which is induced LH. Cycloheximide does not prevent LH from initiating luteinization even at concentrations which are effective in blocking the steroidogenic response of corpora lutea to LH.

Studies on the inhibition of ovulation with prolactin suggest that the anti-ovulatory action of prolactin is secondary to maintenance of progesterone secretion from the corpora lutea. A pronounced inhibitory influence of prolactin on the conversion of progesterone to two major metabolites, tentatively identified as 20α-hydroxy-pregn-4-3-one and 5α-pregnan-3α,20-diol has been demonstrated.

Intracellular mechanisms involved in experimentally-induced luteolysis are under investigation. Data presented by the investigator suggest that the decreased conversion of cholesterol to progesterone may cause accumulation of cholesterol at its site of synthesis. In addition, studies relating to the metabolism of progesterone in the female reproductive tract in relationship to its biologic activity are in progress. Results indicate that 5α-pregnan-3,20-dione may be a mediator of physiologic actions of progesterone at the uterine nuclear level. Prolactin was shown to have an extra-ovarian effect on vaginal mucification in the rat.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2193
Contract Title: Hypothalamo-Hypophysial Factors in Reproduction
Contractor: Medical College of Ohio at Toledo
Money Allocated: $82,911 (FY 69 Funds), $70,555 (FY 70 Funds), $56,700 (FY 71 Funds) for 03 year.

Objectives: The principal objective of this research is to develop a reliable and sensitive displacement assay for oxytocin for the purpose of eventually measuring blood levels of this peptide during the menstrual cycle and following coitus. Using this technique, the investigator is assaying oxytocin levels in animals during the estrous cycle and after mating and will eventually apply the method to determining blood levels of this hormone in women. In addition, the structural elements of the oxytocin-binding peptide are being examined and analogues of oxytocin are being synthesized and compared with oxytocin with regards to their ability to bind to the binding peptide.

Major Findings: The amino acid sequence analysis of the hormone-binding polypeptide of the posterior pituitary has been completed. There are ninety-two amino acids with an N-terminal alanine and C-terminal leucine. The molecule has fourteen cysteine residues but locations of disulfide bridges have yet to be ascertained. Attempts are presently underway to prepare crystalline material for X-ray crystallography. In the meantime, portions of the molecule will be examined by model building for conformity with the requirements for binding oxytocin.

The affinity of the polypeptide for oxytocin was found to be too weak for practical use as the basis for an oxytocin assay. A search is therefore being instituted for a more avid binder in the lactating mammary gland.

Several new analogues of oxytocin 4-threonine analogues have been prepared. The 4-threonine oxytocin was shown to be more potent oxytocic agent than oxytocin itself which may result from a more efficient binding to receptor sites in the uterus.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to support research directed towards the elucidation of the hypothalamo-hypophysial control of reproductive function.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2194
Contract Title: The Role of Progesterone and Other Gonadal Hormones in the Initiation and Maintenance of Early Pregnancy
Contractor: Washington University
St. Louis, Missouri
Money Allocated: $46,329 (FY69-Funds) $48,718 (FY 70 Funds)
$48,718 (FY 71 Funds) for 03 year

Objectives: This investigation involves three projects: 1) Removal of corpora lutea in early human pregnancy and observations of plasma progesterone levels, uterine activity and abortion. Progesterone determinations are also made on the enucleated corpora lutea and the placenta. For patients coming to surgery after the first half of the first trimester, pregnancy is terminated by the intra-amniotic injection of hypertonic saline and the effects of this treatment on plasma progesterone and uterine activity are studied. 2) The role of placental estrogen in luteal maintenance is being studied in rabbits and rats with emphasis on questions of timing, dose, duration in treatment and species differences. 3) Electrophysiological studies, being conducted on the rabbit uterus and oviduct under a variety of hormonal conditions include: tension measurements in electrically stimulated uterine and oviduct strips; measurements of differential shortening of stimulated muscle strips; and direct measurements of action potentials to establish the extent and velocity of conduction in uterine and oviduct muscles. These studies will be extended to observations of the human Fallopian tube.

Major Findings: The investigator performed luteectomy during the first trimester of pregnancy on eleven patients and measured the diameter of the corpus luteum, the uterine size, peripheral plasma progesterone levels, plasma 20α-OH progesterone levels, intra-uterine pressure, and oxytocin response. The findings were compatible with the concept that it is progesterone which is indispensable in pregnancy maintenance and that the human corpus luteum is only indispensable as long as it is the major source of progesterone. Subsequent clinical trials in a group of ten patients verified the expectation that luteectomy would terminate pregnancy at and before the seventh week of gestation (when the corpus luteum is flourishing).

Studies on the effect of estrogen on luteal progesteronegenesis indicate that estrogen replacement therapy, in placental dislocated rabbits, prevent the involution of the corpora lutea, the precipitious decrease in luteal, plasma and uterine progesterone levels, the evolution of intra-uterine pressure, oxytocin response and abortion. Apparently estrogen stimulates luteal function in pregnant rabbits.

Contract Number: 69-2194 (page 2)

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the importance of the corpus luteum in early human pregnancy and the timing of the luteal-placental shift.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2207
Contract Title: Prolactin Production from Primate Pituitary Organ Cultures
Contractor: Boston University
Money Allocated: $5,370 (FY 69 Funds), $22,318 (FY 70 Funds)

Objectives: Rhesus monkey glands cultured in vitro continue to secrete prolactin whereas the secretion of other hormones ceases. This prolactin is being isolated and purified by preparative disc electrophoresis and antisera against it obtained for the development of a specific radioimmunoassay for primate prolactin.

Major Findings: The investigator performed a total of three monkey anterior pituitary (AP) cultures and one human AP culture and established methods that result in the survival of primate AP tissue in organ culture with continued production of prolactin activity for greater than 100 days. Prolactin activity of animals submitted to hemorrhage prior to AP culture was fairly constant at 16-20 IU/ml/100 gm tissue. If the animals were not subjected to hemmorrhage stress, prolactin activity fell from 16 IU to 9 IU between day 1 and day 2 and then remained fairly constant. Initial prolactin production was higher if 95% O_2-5% CO_2 was used in the AP cultures.

Analytical disc electrophoresis of monkey and human AP shows five bands at initial culture. Comparing a pattern of human AP and HCG, the investigator presents evidence for identification of a human prolactin band. Densitometric measurements indicate a specific activity of 125 IU/mg for human prolactin calculated on the amount of protein known to be contributable to HCG.

Significance to Biomedical Research and Program of the Institution: Since nothing is known of the role of prolactin in the regulation of ovarian function in primates, information pertinent to this issue is eminently appropriate.

Proposed Course: Terminated

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2208
Contract Title: Luteolysis as an Approach to Contraception
Contractor: The Upjohn Company
Money Allocated: $127,684 (FY 69 Funds) for two years, $73,425 (FY 71 Funds)

Objectives: This is a study of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) in which the conditions under which $PGF_2\alpha$ causes luteolysis and induces the termination of pregnancy will be determined, and the mechanisms by which $PGF_2\alpha$ achieves its luteolytic effect will be identified. Pregnancy termination is being studied in rabbits, guinea pigs, and rhesus monkeys by ovarian histology and ovarian and peripheral plasma progesterone levels. The period of pregnancy subject to interruption by $PGF_2\alpha$-induced luteolysis is being established.

The second aspect of the proposal is an attempt to study the processes involved in the luteolytic effects of $PGF_2\alpha$. This includes studies of effects on gonadotrophin activity, competitive inhibition of gonadotrophins, direct effects on progesterone synthesis in the ovary by measurement of steroid levels in ovarian venous blood, and of the effects of prostaglandins on steroidogenesis by rat ovaries and primate corpora lutea in vitro. This also includes studies of cholesterol incorporation into progesterone, levels of adenyl cyclase, inhibition of effects of puromycin and cycloheximide, and potentiation of effects by phosphodiesterase inhibitors.

Major Findings: The investigators have demonstrated that $PGF_2\alpha$ was luteolytic in rhesus monkeys when injected subcutaneously b.i.d. for five days if injections were initiated on day 11, 12 or 13 post ovulation of fertile cycles. During later stages of pregnancy (between day 30 and 127) in the monkey PGE_2 is about ten times as potent as $PGF_2\alpha$ in causing uterine contractions and abortions. Prostaglandins, therefore, are effective in terminating pregnancy in the monkey either during or after the period when the corpus luteum is essential for pregnancy maintenance.

Studies on the effect of prostaglandins on ovarian and placental steroidogenesis and on interaction with gonadotropins indicate that ova release in FSH primed immature rats by HCG administration was reduced in the presence of prostaglandin.

In the hamster, prostaglandins interrupted pregnancy most effectively during nidation, a time when progestational steroids are required for implantation and when myometrial activity might be expected to have a deleterious effect on the conceptus.

Contract Number : 69-2208 (page 2)

Significance to Biomedical Research and Program of the Institute: The relevance of this study to the contract program is direct, particularly insofar as some of the work is being done in primates

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2209
Contract Title: Isolation of a Bovine Uterine Luteolytic Factor
Contractor: Cornell University
Money Allocated: $107,232 (FY 69 Funds); for three years

Objectives: The principal investigator has prepared a bovine endometrial extract with the ability to regress corpora lutea of pseudopregnant, hysterectomized hamsters. The active material precipitates with ammonium sulfate and apparently is a large molecular weight protein; the possibility is being investigated that it may be prostaglandin $F_{2\alpha}$ adsorbed to a protein. These studies involve further fractionation and purification of the active material using preparative disc electrophoresis, and study of its effects on corpus luteum progesterone synthesis stimulated by LH, the effects of various modes of administration, and effects on ovarian blood flow and progesterone concentration.

Major Findings: The major part of the luteolytic activity of bovine endometrial extract is retained in the cytoplasmic protein fraction. This activity is associated with an identifiable band in the gel electrophoresis preparations. This band seems very similar to the α-1 globulin described by Milgram and Baulieu and identified as the progesterone binding protein in the rat uterus. Luteolysis in the hamster has been achieved with as little as 0.5 mg of this protein. This represents about 1/5000th of the total protein extractable from one-mid cycle bovine endometrium and about 1/50th of the active protein found in one bovine endometrium. The cytoplasmic fraction is also luteolytic in the cow.

The luteolytic factor that is lipid soluble does not appear to be prostaglandin $F_{2\alpha}$, but could be a steroid.

Significance to Biomedical Research and Program of the Institute: Corpus luteum regression, or "luteolysis" has been identified as particularly important to contraceptive development; therefore, this study is directly relevant to the purposes of the program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2211
Contract Title: Identification of the Luteotrophic Complex in the Ewe
Contractor: University of Wyoming
Money Allocated: $39,820 (FY 69-70 Funds), $21,252 (FY 71 Funds)

Objectives: The investigator is continuing studies on luteal maintenance in the hypophysectomized ewe. He is attempting to identify the nature of the unknown material in crude LH preparations which, on constant IV infusion, maintains corpora lutea in a functional state in hypophysectomized ewes, while purified LH preparations are unable to do so. Various combinations of ovine LH, FSH, prolactin, TSH, ACTH, and GH are used. Measurements made include twice daily peripheral plasma progesterone levels, and size and progesterone content of corpora lutea removed at the end of the eight-day experimental period.

Major Findings: On the basis of the data collected thus far, it does not appear that crude pituitary extracts, crude LH preparations, or pure LH with or without addition of prolactin are capable of maintaining corpora lutea past the normal time of regression in the non-pregnant ewe if all functional pituitary tissue has been removed. The increasing peripheral levels of prolactin in those animals in which corpora lutea were maintained may indicate that prolactin is necessary for maintenance of corpora lutea in the ewe. Although it is quite clear that the luteolytic effect exerted by the uterus in the ewe can be overridden by infusions of LH in the intact ewe and in ewes with only small amounts of pituitary tissue remaining, it does not appear that this will be accomplished easily in the completely hypophysectomized animal.

Significance of Biomedical Research and Program of the Institute: Although, because of the apparent differences which exist between species, it is not clear that knowledge of the process in sheep will by itself be of assistance in determining the factors maintaining luteal function in the human, it is clear that a more rational approach to the induction of luteolysis can be made if the nature of luteotrophic factors is known.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2212
Contract Title: Site of Blockade of Ovulation by Progestins in the Monkey
Contractor: Tulane University
Money Allocated: $44,000 (FY 69 Funds), for two years, $42,246 (FY 71 Funds)

Objectives: These studies are directed towards establishing the feedback site of progestins as inhibitors of ovulation in the rhesus monkey using experimental approaches previously used in the rabbit. These approaches are feasible in the rhesus monkey but much base-line information is being obtained, preliminary to critical studies of feedback inhibition of ovulation.

Major Findings: The data indicate a six to ten fold increase above baseline value in serum luteinizing hormone (LH) which proceeds ovulation by several hours in the monkeys. Progesterone (0.5, 2.0 or 5.0 ng/day), injected between 8-22 days of the menstrual cycle blocked the LH surge and ovulation. The low dosage of progesterone (0.5 ng/day) injected between days 2-10 or 8-16, but not between day 2-6, of the cycle also prevented the LH surge and ovulation. Estradiol 17-β (100-300 μg) for three consecutive days at the end of the progesterone regimen resulted in ovulation and an LH surge in 3 of 8 monkeys and smaller elevations in LH without ovulation in two others.

Median eminence extracts of sheep origin (SMEE) given systemically produced a 3-100 increase in serum levels of LH. A dose-response curve for 1/50 to 1 SMEE equivalent was established. The LH rise occurred within 5-10 min and returned to baseline within 30 min. Ovulation did not accompany the rapid surge in LH. SMEE produced the LH elevation when given on day 5, 12 or 22 of the menstrual cycle and exogenous progesterone failed to block the rapid LH surge caused by SMEE. Intrapituitary infusion of MEE from sheep or rabbit or of LH-RH has produced only small changes in serum LH as compared with systemic infusions to date.

Significance to Biomedical Research and Program of the Institute: Studies of the mechanism of ovulation are clearly relevant to the purposes of the contraceptive development program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2213
Contract Title: Effects of Lactation and Stress on Ovarian Activity
Contractor: Iowa State University
Money Allocated: $71,267 (FY 69 Funds); for two years

Objectives: These studies are concerned with determining the factor(s) responsible for ovarian quiescence in the lactating cow. The hypothesis being explored is that oxytocin/vasopressin released at milking acts at either the level of the hypothalamus-pituitary, uterus, or adrenal to interfere with follicular development. Experiments to test these relationships include: (1) the effects of infusion of oxytocin or ACTH on plasma cortisol levels and corpus luteum formation; (2) the effects of infusion of adrenal steroids directly on corpus luteum formation post-ovulatory; and (3) determination of possible interplay between ACTH and LH plasma levels. Bioassays will be performed for cortisol, oxytocin, ACTH.

Major Findings: Results of the research indicate that suckling significantly delayed the time of the first postpartum ovulation when compared to non-lactating animals. There was a significant decrease in weight of the previously gravid horn in the suckled cow but there were no differences in weight of the various endocrine glands between suckled and non-suckled animals. Thyroid activity was similar in all groups.

Analysis of plasma samples for corticoids has demonstrated a consistent rise in plasma corticoids following the stimulus of milk removal. Infusion of vasopressin and oxytocin (given through a jugular vein cannula) were utilized to determine their effect on adrenal steroid production. The only significant increase in plasma corticoids resulted from a 10 u. dose of vasopressin although there was a nonsignificant rise following administration of 5 u. vasopressin.

Five cyclic cows were treated with a corticoid (Flumethasone-difluoromethlyprednisolone) on days 1-8 of the estrous cycle. No differences were observed in weight or progesterone content of the corpora lutea.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Terminated

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2214
Contract Title: Contraception Through Disordering of Luteal and Uterine Events
Contractor: Harvard Medical School
Money Allocated: $158,441 (FY 69 Funds) for three years

Objectives: The major aim of this program is to determine the hormonal factors regulating the mammalian corpus luteum - its growth and regression. Experiments being carried out under this contract deal with:

1. the significance of cholesterol esterases in normal, unsuperovulated ovaries during different functional states;
2. development of testing methods for measuring oxygen consumption in intact CL;
3. effects of LH on steroidogenesis in mouse Leydig cell tumors;
4. pituitary luteotropic and luteolytic activity;
5. direct action of progesterone on the ovary;
6. uterine luteolytic activity in guinea pigs;
7. influence of IUD's on CL function.

Eventually, findings in rats and guinea pigs will be tested in primates.

Major Findings: The investigators have shown that the steroidogenic activity of the rat ovary is closely related to the turnover of cholesterol esters and the activity of the enzyme cholesterol ester synthetase. Esterified cholesterol must be hydrolyzed to free cholesterol before being available for steroid synthesis. LH has an action to increase the conversion of esterified cholesterol to free cholesterol within ten minutes of incubation.

Significance to Biomedical Research and Program of the Institute: This is a comprehensive program the objectives of which are directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2215
Contract Title: Relationships of Endometrial Prostaglandins and Luteal Function
Contractor: West Virginia University
Money Allocated: $35,337 (FY 69 Funds) for two years, $32,460 (FY 71 Funds)

Objectives: The investigators are extracting the endometrium of sheep at various phases of the cycle to determine prostaglandins both qualitatively and quantitatively. The effects of progesterone on the corpus luteum and on prostaglandin levels and the effects of prostaglandin $F_{2\alpha}$ on corpus luteum function in hysterectomized animals are being studied. Corpus luteum function is being evaluated by progesterone levels and length of estrous cycle.

Major Findings: A method for the extraction, partial purification and quantification of prostaglandins (PG) from uterine endometrium has been developed. Recovery rates (using tritium labeled $PGF_{2\alpha}$) averages approximately 55%. Very low quantities (as low as 1.8 ng/ml) can be detected by the gerbil colon bioassay. Experiments on mature non-pregnant ewes show higher levels of $PGF_{2\alpha}$ on day 14 of the cycle, and at day 5 after an IUD has been inserted on day 2. Since these are times when luteal regression is beginning to occur, the data supports the possibility that PGF_2 plays a physiologic role in luteal regression. PGE_2 $PGF_{1\alpha}$ and $PGF_{2\alpha}$ can be detected in endometrial preparation; PGE_1 was not detected.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the factors responsible for the control of corpus luteum function.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2216
Contract Title: Effect of Progestational and Estrogenic Hormones on the Uterus
Contractor: Albert Einstein College of Medicine
Money Allocated: $124,274 (FY 69 Funds) for two years, $62,137 (FY 71 Funds)

Objectives: The main objective of this research project is to determine the role of progesterone in the biochemical and morphologic changes in the rabbit endometrium and myometrium which take place following ovulation. Principal parameters being studied include cytochrome content of mitochondrial and microsomal fractions, total DNA, RNA and protein, uptake of labeled precursors, O_2 uptake, and endometrial enzymes. Appropriate biochemical and ultrastructural techniques are being applied to these measurements.

Major Findings: Studies on the effects of progesterone and 17β-estradiol demonstrate a striking difference. Estrogen appears to stimulate the entire uterus in a similar manner not differentiating between endometrial and myometrial cells as far as the total nucleic acid and protein contents of these tissues are concerned. In contrast progesterone stimulates DNA synthesis in the endometrium but not to any appreciable extent in the myometrium.

With estrogen treatment, the synthesis of an "induced protein" reported by other investigators appears to take place in the rabbit endometrium.

Cells from hormone stimulated endometrium have been separated into three types, examined in the electron microscope, and maintained in culture.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to determine the mechanism of action of ovarian hormones on uterine metabolism and morphology.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2217
Contract Title: Effect of Autonomic Drugs on FSH and LH Releasing Factors
Contractor: The Ohio State University
Money Allocated: $41,887 (FY 69 Funds) for three years

Objectives: The objective of this research project is to study the release of LH by pituitary halves incubated with median eminence slices and hypothalamic minces, and to evaluate the inhibitory effects of various drugs. In a second series of experiments drugs will be injected into the ventricular system of the rat brain and LH release evaluated.

Major Findings: Experiments performed to date indicate that more than one biogenic amine and more than one cholinergic pathway is involved in modulation of FSHRF and LHRF in ovariectomized rats. Thus, in the case of FSH release, two biogenic amines DOPAmine and norepinephrine, have opposite effects, the former stimulating and the latter inhibiting release. Two different types of cholinergic blockade also have opposite effects on FSH release. Changes in LH content of plasma and pituitary were much less pronounced and differed in other ways from changes in FSH content following treatment with agents which affect neurotransmitter mechanism.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2221
Contract Title: Physiological Aspects of the Attachment of Carbohydrate to Luteinizing Hormone
Contractor: University of Texas
Houston, Texas
Money Allocated: $119,340 (FY 69 Funds) for three years

Objectives: The investigator is collecting sheep pituitary glands and classifying them according to sex or age of the animal in order to determine whether these parameters affect the carbohydrate subunit and its attachment to the luteinizing hormone molecule. Various theoretical considerations have led to the postulate that LRF activity may be defined as the process of activating a particular type of carbohydrate attachment to the protein core of LH in the pituitary. The data being collected in this study will go far toward testing this postulate. This program is of interest in connection with studies of ovulation control and corpus luteum formation, in addition to its primary importance in helping to clarify the chemistry of luteinizing hormone.

Major Findings: The investigator has accomplished the separation of the subunits of luteinizing hormone and have characterized the glycoprotein moieties. Another major finding has been the elucidation of the amino acid sequence of LH. Preliminary studies suggest that the subunits may provide information on the mechanism of action of the hormone.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the mechanism of action of the hypothalamic-releasing factors and the pituitary hormones.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2140
Contract Title: Fertility Control and Contraception
Contractor: Cornell University Medical College
New York, New York
Money Allocated: 52,305 (FY 70 Funds)

Objectives: The objective of this research project is to measure plasma concentrations of testosterone, progesterone, androstenedione, pregnenolone, dehydroepiandrosterone, FSH and LH in the menstrual cycle, both normal and abnormal, and in the puerperium, in post-menopausal women and in prepubertal, pubertal, and post-pubertal males. The investigator wished to establish normal ranges, variations, and time sequences. Both peripheral and gonadal blood levels of steroids will be determined. In addition, FSH and LH levels, metabolic clearance rates and production rates of FSH and LH in relation to hormonal secretion will be studied.

Major Findings: Data are provided to show that an appreciable surge in testosterone and androstenedione in boys occurs about age of 10. Plasma levels of dehydroepiandrosterone and its sulfate ester in both boys and girls increases at about 8 years of age. These findings suggest that there is some change in adrenal steroid secretion prior to and its sulpate. The investigators have set up procedures for the radioimmunoassay of E^1, E^2, and E^3 and have carried out E^2 analysis on polled plasma samples. The values obtained were as follows: (1) pooled male plasma 21±3 pg/ml (15 samples); female plasma 54 + 2.4 pg/ml. In children under 5 the value was 7 pg/ml and in post menapausal women 11 pg/ml. Hirsute women during luteal phase had E^2 values of 235 pg/ml, whereas normal follicular phase values were 49 pg/ml.

In a patient undergoing hypophysectomy, there is a rapid decrease in plasma level of both gonadotropins. Administered FSH disappears from blood in about 45 minutes.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies devoted to describing quantitatively the hormonal components of the normal human menstrual cycle.

Proposed Course: Terminated

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract amd Collaborative Research

Contract Number: 70-2145
Contract Title: Progesterone Production and Metabolism During the Human Female Reproductive Cycle
Contractor: Case Western Reserve University
Cleveland, Ohio
Money Allocated: $30,430 (FY 70 Funds), $34,711 (FY 71 Funds)

Objectives: The first objective of this project is to study the plasma metabolic clearance rate and plasma production rate of progesterone and 20α-hydroxypregn-4-en-3-one in human subjects at the mid-follicular phase, the mid-cycle period, and the mid-luteal phase of the menstrual cycle. These values will be correlated with plasma levels of luteinizing hormone as measured in the same patients and the percent conversion of progesterone in vivo to 20α-hydroxypregn-4-en-3-one will be calculated. Competitive protein binding methods will be employed to measure plasma concentrations of both steroids.

A second aspect of this research involves evaluation of the influence of plasma protein binding on the metabolism and distribution of progesterone. To study the effect of steroid binding to transcortin on the steroid distribution and metabolism, monkeys will be infused with tritiated progesterone, either in the absence or presence of purified human transcortin. In addition to measuring metabolic clearance rate in these animals, hepatic extraction of free and bound progesterone will be measured in monkeys with catheterized hepatic veins. It is also proposed to study the influence of transcortin on the uptake of tritiated progesterone from the plasma by a variety of tissues, in rats. Metabolic products of progesterone found in these tissues will also be measured.

Major Findings: The plasma metabolic clearance rate (MCR) has been measured in 12 women at various times during menstrual cycle. Progesterone was not associated with blood cells whereas with 20α-hydroxy progesterone there is about 20-25% that is associated with blood cells.

It was observed that a 2 hour infusion rate of labelled progesterone was long enough to achieve the plasma isotopic steady state for progesterone and to achieve the plasma isotopic steady state for ^{3}H-20-α-hydroxy- progesterone derived from ^{3}H-progesterone.

The MCR for progesterone does not appear to exhibit a set pattern during the menstrual cycle, likewise the conversion of progesterone to 20α-hydroxy progesterone tends to fluctuate considerably (5-15%). In a single subject the conversion rate tends to double from mid-follicular to the mid-luteal phase and is in line with the increased plasma progesterone concentration.

Contract Number: 70-2145 (page 2)

New methodology for 20-α-hydroxy progesterone measurement has been developed.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of ovarian steroid metabolism and production during the menstrual cycle in primates including humans.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2149
Contract Title: The Control of Ovulation and Corpus Luteum Function
Contractor: Medical College of Georgia
Money Allocated: $43,760 (FY 70 Funds), $25,891 (FY 71 Funds)

Objectives: This research project involves investigations in two areas: (1) the role of FSH and LH in induction of ovulation and regulation of luteal function; and (2) the nature of steroid-protein interaction in the hypothalamus and pituitary.

In the first area, factors responsible for the ovulatory surge of gonadotropins will be studied by bilateral ovariectomy on the day before proestrus, and replacement therapy with estrogens and/or progesterone to restore the normal surge of ovulatory hormones. Endpoints will be pituitary FSH and LH measured by bioassay and serum FSH and LH measured by radioimmunoassay. Initially, the work will be conducted in hamsters; it will later be extended to rats and guinea pigs. The role of FSH and LH in corpus luteum function will be studied in phenobarbital-blocked immature rats and hamsters primed with PMS. Ovulation is then induced with FSH and/or LH and corpus luteum function evaluated morphologically and by monitoring progesterone levels in the ovarian venous effluent and peripheral plasma.

The second part of the proposal applies the classical techniques developed to identify uterine receptors for estrogen to the hypothalamus and pituitary. This includes studies of the uptake, retention and relative rates of metabolism of injected radio-labeled estradiol by the hypothalamus and pituitary and the use of stereotaxic lesions to localize binding sites. Further studies will explore the effects of estradiol on RNA, phospholipid, and protein synthesis by the hypothalamus and pituitary, and on gonadotropin synthesis and release. Purification and study of specific binding components will be undertaken later in the work.

Major Findings: It has been shown that estradiol is firmly bound to a specific macromolecular component from female and male rat pituitaries. Testosterone was not preferentially bound. Pituitary cytosol reaction from male rats did bind estradiol, but not testosterone. Dihydrotestosterone is bound at an intermediate level between estradiol and testosterone. These results indicate that a common receptor protein exists in the cytoplasm of male and female rats and suggests a biological role of estradiol in the pituitary, exclusive of its role in the hypothalamus. Data for the hypothalamus are similar to the pituitary data.

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2149 (page 2)
Contract Title: The Control of Ovulation and Corpus Luteum Function
Contractor: Medical College of Georgia

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the action of the pituitary gonadotropins in the reduction of ovulation and the control of corpus luteum formation, function, and regression.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program..

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2158
Contract Title: The Development of Inhibitors to Estrogen Biosynthesis
Contractor: Worcester Foundation for Experimental Biology, Inc.
Money Allocated: $43,634 (FY 70 Funds), $43,634 (FY 71 Funds)

Objectives: In previous work the investigator has shown that the conversion of androst-4-ene-3,17-dione and testosterone to estrogens involves reaction sites at carbon 1, 2, and 19, and that these sites are not utilized in other important steroid metabolic processes. Based on these findings, this research project is devoted to developing specific inhibitors to the aromatase enzyme system which catalyzes the production of estrogens. The investigator seeks a specific, effective inhibitor which may result in lowered estrogen levels in vivo. The enzyme system will be prepared from human placentas utilizing a standard preparation with appropriate controls.

Major Findings: Working on the hypothesis that compounds structurally similar to the natural substrate could inhibit the aromatization reaction they have synthesized and tested a number of compounds related to the androstane and estrane ring structures. The inhibition of the conversion reaction of androstenedione to estrone and estradiol is carried out in vitro utilizing human placental microsomes as the source of the enzyme. In general, structures that were good inhibitors of estrogen biosynthesis were structurally related to the substrate. Several compounds notably 1α, 17β-dihydroxy-Δ^4-androsten-3-one, Δ^4-estrene-3,6,17-trione and 5α-androstane-3,17-dione were good inhibitors and appear to bind as well to the estrogen producing "aromatase" enzyme system as the known substrates for estrogen biosynthesis.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the biosynthetic pathways for ovarian steroids with special attention being paid to possible points of inhibition for the purpose of fertility control.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2160
Contract Title: The Role of Uterine Luteolytic Substances in Reproduction: An Evaluation of the Specificity of Porcine Granulosa Cell Monolayers as an Assay System
Contractor: Duke Univeristy Medical Center
Money Allocated: $17,070 (FY 70 Funds) $17,070 (FY 71 Funds)

Objectives: This investigator's previous finding that uterine flushings of pigs obtained during luteal regression rapidly destroy granulosa cells cultured in vitro, whereas flushings from the earlier portions of the estrous cycle do not, has been criticized because the specificity of this lytic action has not been established; on the contrary, there is evidence that it may be quite non-specific. The investigator now proposed to undertake a through study of the specificity of his cell culture assay system. Extracts from various tissues as well as a number of enzymes will be used. Conversely, the activity of uterine extracts in cell cultures of various non-ovarian tissues will also be examined. Lytic activity will be evaluated by time-lapse cinematography.

Major Findings: Using Sephadex G-200 columns the material from the uterine flushings was characterized as a high molecular weight substance. Studies of tissue extracts from skeletal muscle, heart, liver, spleen, pancreas, lung, ovarian interstitium and kidney did not produce a similar degree of lysis as did the luteal phase uterine flushings. A number of enzymes, progesterone, estradiol 17β and $PGF_{2\alpha}$ could not duplicate data obtained with flushings. Extracts of endometrium were not lytic when the amount of extract protein was held to 10 mg/culture. At higher concentrations of protein, endometrial extracts were highly lytic. Flushings on the other hand were lytic when added to culture at the equivalent of 1.5 mg/culture. The amount of lysis induced by endometrial flushings was quantitatively diminished when fetal calf serum was substituted for pig serum in the culture medium.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies devoted to elucidating the factors responsible for luteal regression.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2250
Contract Title: Development of Radioimmunoassays for Rhesus Monkey Gonadotropins
Contractor: Endocrine Laboratories of Madison, Inc.
Money Allocated: $78,553 (FY 70 Funds), $78,553 (FY 71 Funds) for 02 year

Objectives: This proposal is based on the recognition that rhesus monkey gonadotropic hormones do not cross-react immunologically with their human counterparts or at least not sufficiently to permit their detection in body fluids by radioimmunoassay. In this project, the investigators will collect urine from gonadectomized rhesus monkeys for the isolation of monkey LH and FSH. The urinary gonadotropins will be utilized because of the limited availability of monkey pituitary glands. The gonadotropins thus obtained will be used as antigens to obtain antisera for development of radioimmunoassays. Purification of the hormones will be by procedures previously developed, and by adaptations of methods developed in other laboratories and reported in the literature.

Major Findings: The work has been concerned primarily with 1) stock-piling extract of urines from ovariectomized monkeys; 2) exploration of assays of a pooled rhesus monkey urine extracts in comparison with a number of other gonadotropin assays for LH, FSH and total gonadotropins; 3) variation in the extraction procedure to see what are the optimal conditions for the recovery of FSH and LH.

Initial assays of individual monkey urines has allowed the separation of the ovariectomized colony into 4 groups: high LH, medium LH, high FSH and medium FSH. The urines were collected under refrigeration, acidified and then stored at -20°C. The two primary procedures that have resulted in large quantities of crude gonadotropins have been the kaolin-acetone extraction procedure and the ultrafiltration procedure. A total of 1,468 liters of urine have been extracted with anticipated activity equivalent to 275 to 300 mg of NIH-FSH and total LH activity equivalent to approximately 40 to 50 mg of NIH-LH. Comparing different FSH assays the investigators have concluded that the Steelman-Pohley assay was most advantageous. In testing the potency of extracts it appears advisable to acidify during collection if the urine is to be used for FSH extraction.

For LH assays the investigators used the OAAD test as described by Bogdanove and Gay. Using this assay it was determined that kaolin-acetone extraction and ultrafiltration recovered approximately equivalent amounts of LH activity. The direct acetone precipitation, and direct acetone precipitation plus kaolin-acetone extraction, appears to yield approximately three to ten times that obtained by kaolin-acetone extraction.

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July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2250
Contract Title: Development of Radioimmunoassays for Rhesus Monkey Gonadotropins
Contractor: Endocrine Laboratories of Madison, Inc.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of new methodologies for the assay of steroid and protein hormones in the blood of primates including man.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2251
Contract Title: Pilot Study for the Large Scale Preparation of Purified Human Chorionic Gonadotropin
Contractor: Columbia University
New York, New York
Money Allocated: $47,300 (FY 70 Funds), $85,086 (FY 71 Funds)

Objectives: The objective of this investigation is to undertake a feasibility study to determine the optimal conditions for preparation of large batches of highly purified human chorionic gonadotropin (HCG). Secondary goals of this program are: (1) to obtain data bearing on the chemical and immunological characterization of HCG fractions which appear to be homogeneous in polyeptide content, but possess differing biological activities; and (2) to obtain data on the stability of HCG using both immunological and biological activities as guides.

Major Findings: Two batches of crude HCG were purchased from Organon and Roussel. Each batch contained 50,000,000 IU. The purification set-up can handle about 10,000,000 IU or 3 to 4 gms of starting material at one time. The steps employed are as follows:

A. Batch absorption of HCG to sulphoethyl Sephadex
B. Gel filtration through Bio-Gel p-150
C. Ion exchange chromatography on DEAE-Sephadex

The recovery of active material in step A is about 80% which is within the errors of the bioassay procedure. Recovery of activity in step B is also very high. There is some loss of active material during the last step. Overall from 4g of starting material there is a recovery of 0.4g of highly active material. The total recovery of biological activity is about 40%. Variation in the procedure did not essentially improve recovery and the sequential steps are presently recommended for preparation of large batches of highly purified HCG.

Repeated lyophization of the purified material did not reduce biological activity. Other stability experiments are planned.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of predecures for human hormone isolation and purification.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2255
Contract Title: The Biochemical Life Cycle of the Rabbit Corpus Luteum and Its Modification by Humoral Factors
Contractor: Medical University of South Carolina
Money Allocated: $21,493 (FY 70 Funds), $21,493 (FY 71 Funds)

Objectives: The life cycle of the rabbit corpus luteum will be studied with respect to several biochemical parameters, all of which are related to the ultimate function of this organ, elaboration of progestagens. The initial parameter to be investigated is adenyl cyclase activity which will be studied in the rabbit corpora lutea at varying times after ovulation and luteinization have been induced by the administration of luteinizing hormone (LH). Adenyl cyclase will be determined in a particulate fraction from homogenates by the conversion of labeled ATP to cyclic-AMP.

Major Findings: Corpora lutea varying in age from 2 to 6 days have been assayed for adenyl cyclase activity. The data indicate that there is an increase in activity from days 2 to 6.

Ratios between the control and fluoride stimulated activities and between the LH stimulated and fluoride stimulated ones do not show any consistent change with age of the corpora lutea. This finding suggests that, if there is a special LH responsive type of adenyl cyclase, it varies with age of the corpus luteum in the same manner as the non-specific type. Perhaps a more likely explanation is that only one type of adenyl cyclase is present in corpora lutea and that it is responsive to LH.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the biochemical mechanisms involved in corpus luteum function.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2256
Contract Title: Studies on the Mechanism of Luteinization *In Vitro*
Contractor: University of Pittsburgh
Money Allocated: $25,216 (FY 70 Funds)

Objectives: This investigation involves studies on the mechanism of luteinization to be carried out *in vitro* using cultures of rhesus monkey and porcine granulosa cells. The time of exposure of granulosa cells to LH and FSH necessary for luteinization will be examined and compared to the effects of N^6-2'-O-dibutryl-cyclic-3',5'-AMP. Each agent will be added and subsequently removed after various time intervals and effects will be assessed in terms of morphology, progesterone and estrogen secretion and luteinization. The effects of LH and FSH on adenyl cyclase activity in porcine granulosa cells and possibly monkey cultures will be examined to confirm or disprove the concept that cyclic AMP is a luteinization mediator. Prostaglandins will be added to cultures of procine and monkey granulosa cells to determine their effects upon luteinization. If possible, effects on adenyl cyclase will be examined also.

Major Findings: Granulosa cell cultures from monkey ovaries respond to the *in vitro* addition of LH by increased progestin secretion. Addition of human FSH to the culture produced a response similar to that of LH. Addition of either gonadotropin had to be done at early stages of culturing for if the addition was done on day 10 or later of culture there was no significant stimulatory effect on progestin secretion.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies on the mechanism of luteinization and its control by hormonal environment.

Proposed Course: Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2260
Contract Title: Mechanisms of Progesterone Action on the Central Nervous System
Contractor: Michigan State University
Money Allocated: $20,300 (FY 70 Funds), $20,300 (FY 71 Funds)

Objectives: The objective of this research program is to identify and study progesterone receptor regions in the central nervous system of the rat. A progesterone receptor region which is related to sexual receptivity has recently been located in the mesencephalic reticular formation. It is proposed here that studies be undertaken to determine the neurobiochemical systems involved in progesterone action at this site and that work be initiated to determine the relationship of these receptors to ovulation. In addition, work is proposed to explore other regions of CNS for progesterone receptor sites which are involved in the regulation of ovulation. Techniques to be used include the standard series of steroid implants at various regions of the brain.

Major Findings: The investigator has observed that the local strain of rats (Holtzman) was not suitable for the study of the effects of progesterone on the lordosis response in ovariectomized estrogen primed rats. In these animals progesterone was delivered to the mesencephalic reticular formation (MRF) by means of brain cannula. The lordosis index was high in over 50% of the animals given estrogen. The variable response observed with a variety of estrogen schedules prevents formulation of a stable estrogen baseline. Work is being repeated with another strain of rats.

In another set of experiments the investigator checked the effect of progesterone implants on ovulation delay in the 4-day cycling rat. Cannulae were inserted into MRF, hypothalamus (HYP) and the preoptic area (POA) and progesterone implanted on the 1st day of diestrus (11-12a.m.) and removed 4 hours later. Oviducts were checked for ova at 72 and 96 hours after implant removal. Progesterone in MRF blocked ovulation in 4 out of 13 females (30%), implant in HYP blocked 5 out 9 (55%). Females with implants in POA appear to ovulate normally.

In the 5-day cycling rat results were inconclusive.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the feedback site of steroids in the central nervous system.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2307
Contract Title: A Simultaneous Theroretical and Empirical Approach to the Study of the Rat Estrous Cycle
Contractor: University of Illinois
Money Allocated: $41,500 (FY 70 Funds), $45,226 (FY 71 Funds)

Objectives: The investigator plans a systems analysis approach to the understanding of the mechanisms regulating reproductive cyclicity and acyclicity in the rat. The total approach includes the following stages to be carried out simultaneously: identify from existent experimental data the essential variables and connecting linkages among system components; make a model of the system; simulate the system by computer; perform "experiments" using the model and simulating by computer to compare the model to similar experiments in the real system; use the computer to simulate the results of new experiments; perform these experiments and modify the model if necessary, etc.

Because data on few of the variables needed for constructing an adequate simulation are available from the literature, the investigator will measure the following parameters and variables using radioimmunoassay and competitive protein binding methods for steroids and gonadotrophic hormones; (1) estrogen, progesterone, LH, FSH, and prolactin in serum throughout the four-day cycle; (2) distribution volumes and loss rate constants of the above hormones in cyclic rats and following ovarectomy and/or hypophysectomy; (3) setpoints for LH, FSH and prolactin in prepubertal and adult rats; (4) the negative feedback constant for the suppression of LH and FSH secretion by estrogen; (5) ovarian transfer functions, determining the effects of gonadotropins on estrogen and progesterone secretion and on morphology; (6) estrogen, LH, and FSH levels during the onset, steady state and termination of persistent estrus due to continuous light.

Major Findings: Progress has been achieved in the following areas:
a) Computer simulation studies - some changes in the programs had to be undertaken to equate the simulation with the known physiological values that have been obtained. The programs are designed to simulate known physiological events such as ovulation, hormone surges, follicular atresia and others.
b) Measurement of serum LH during the critical period of proestrus in 4 and 5 day cycling rats - it appears that the LH surge in the 4 day cycling rat occurs earlier than previously estimated (16 hrs+) and the 4 and 5 day cycling rats have different patterns.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2307
Contract Title: A Simultaneous Theroretical and Empirical Approach to the Study of the Rat Estrous Cycle
Contractor: University of Illinois

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the mechanisms regulating reproductive cyclicity and acyclicity.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2308
Contract Title: Solid-Phase Radioimmunoassay of Hormones
Contractor: Battelle Memorial Institute
Columbus, Ohio
Money Allocated: $21,165(FY 70 Funds), $21,165 (FY 71 Funds)

Objectives: This is a contract to develop solid-phase radioimmunoassay of pituitary and steroidal hormones in plasma. The investigator will purify HCG, and utilize it to test a solid-phase system based upon covalent attachment of antibodies to polystyrene tubes. The solid-phase antibody will replace the antisera and the double-antibody used in currently operating methods. Conditions for optimal measurement will be worked out and compared with these techniques. In the case of the steroid hormones, a derivative of the particular steroid will be coupled to an antigenic protein and the antibodies produced by this conjugate coupled to polystyrene tubes.

Major Findings: a) Irreversible binding of antisera to a surface
Original work has been done with the binding of I^{131} albumin to polystyrene and polypropylene tubes. Most of the present work deals with glass tubes. To effect attachment, glass tubes were first exposed briefly to γ-aminopropyltriethoxy silane (Union Carbide A-1100), incubated with the antibody, and then reacted with glutaraldehyde. In this reaction sequence an amine group is covalently attached to the glass surface through reaction with the amino silane compound. This amine can then be coupled with free amine groups on the protein molecules by reaction with glutaraldehyde.

b) Reproducibility of the quantity of antisera bound per unit area. The investigator has observed that by limiting the amount of antiserum available for binding he could achieve uniform distribution of antiserum among glass tubes and within the separate sections of the same tube.

c) Retention of antiserum specificity. In attaching the antiserum to the glass surface care must be taken not to block the antigen reactive sites. Glass capillary tube surfaces appear to have a number of desirable features for antibody attachment, including replicate dimentional characteristics, variety of available sizes, possible advantages in antigen binding rates stemming from the use of a high surface area to liquid volume ratio. It has been demonstrated the antigen is bound to the fixed antiserum.

Contract Number: 70-2308 (page 2)

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on radioimmunoassays of hormones.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2310
Contract Title: Regulation of the Changes in Corpus Luteum Physiology Required for the Establishment of Pregnancy in the Rat
Contractor: Case Western Reserve University
Money Allocated: $53,313 (FY 70 Funds), $53,313 (FY 71 Funds) for 02 year

Objectives: The proposed experiments are designed to shed further light upon the interaction between pituitary LH and prolactin and a placental luteotrophic hormone in the regulation of the rat corpus luteum. Interaction between these agents is assumed to be responsible for differences between the corpus luteum of pseudopregnancy and the corpus luteum of pregnancy, the latter being characterized by (a) increased size, (b) ability to maintain increased peripheral plasma levels of progesterone and (c) ultimate development of independence, i.e., the ability to function in the absence of continued support from the pituitary placenta. The fundamental hypothesis to be tested is that in the non-pregnant state LH has a luteolytic action, and the addition to the system of the placental luteotrophic hormone converts this action to a synergistically luteotrophic action.

Experiments will be carried out in the pregnant rat on three principal problems: (1) increase in size of the corpus luteum of pregnancy, (2) elevation in plasma progesterone levels, and (3) development of corpus luteum independence in the absence of pituitary and placenta. Methods to be used include various surgical manipulations, progesterone determination by competitive binding assays, and prolactin determination by radioimmunoassay.

Major Findings: Serum progesterone values during rat pseudopregnancy peak between days 4 and 6 and fall to a minimum on day 10. During pregnancy values on day 5 are the same as during pseudopregnancy, but then they keep on increasing until day 15 of pregnancy.

The next series of conducted experiments were aimed at showing that the CL of pregnancy acquires "independence" from the pituitary and the uterus after 12 days of pregnancy. The measure of this "independence" is the ability of the CL to secrete significant amounts of progesterone in the case when the pituitary and the entire uterus are removed on day 12. The importance of this finding is that it does not occur in pseudopregnancy and probably not during lactation. It, therefore, appears to be almost certainly an effect of placental LTH.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on corpus luteum physiology.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2310
Contract Title: Regulation of the Changes in Corpus Luteum Physiology Required for the Establishment of Pregnancy in the Rat
Contractor: Case Western Reserve University
Money Allocated:

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2312
Contract Title: Metabolism of Ovarian Hormones in the Rhesus Monkey
Contractor: University of Pennsylvania
Money Allocated: $32,867 (FY 70 Funds), $32,867 (FY 71 Funds)

Objectives: The investigator plans a systematic study of ovarian steroid metabolism in the rhesus monkey. The study includes measurement of the progesterone and estrogen levels of peripheral and ovarian venous plasma, measurement of urniary estrogens, injection of radiolabeled progesterone and estrogens to determine production rates and urinary metabolites, and studies of biosynthetic pathways in the ovary. The gathering of this information is preparatory to ascertaining the hormonal requirements for successful conception and gestation and to utilize these in the devising of possible points of attack for contraceptive measures.

Major Findings: Tissue incubations. Corpora lutea of different stages and follicular tissues were incubated in vitro. Radioactive precursors used were acetate-C^{14}, pregnenolone-H^3 and progesterone-C^{14}. The influence of LH, FSH and HCG on the rate of acetate-C^{14} incorporation were also studied. Luteal tissue formed estradiol-17β, estrone, androstenedione, 20-hydroxy preg-4en-3, 20 dione and 17α-hydroxyprogesterone from acetate. The major steroid found in all corpus luteum incubations was progesterone. Follicular tissue formed estradiol, estrone, pregnenolone dehydroepiandrosterone, androstenedione, and progesterone from acetate. LH and HCG stimulated luteal formation of progesterone from endogenous sources and from acetate-C^{14}. Ovarian stromal tissue from anovulatory monkeys incubated in vitro with acetate-C^{14} yielded primarily androstenedione. Estrogens were not found.

Urinary studies. Monkeys in the follicular and luteal phases were given intravenous injections of radioactive estradiol, estrone, androstenedione and testosterone. Estrone glucuronide is the major urinary estrogen. The 16 hydroxy epimer of estriol represents a significant urinary metabolite of both estrone and estradiol. Free estriol and estradiol appear only as minor constituents. Androstenedione but not testosterone was metabolized to estrone.

Progesterone injected into a monkey during the luteal phase yielded primarily androsterone in urine. Pregnanediol epimers are not significant metabolites in the rhesus.

Methodology: Radioimmunoassays have been developed for plasma measurement of estradiol, estrone, testosterone and dihydro-testosterone.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on metabolism of ovarian hormones.

Contract Number: 70-2312 (page 2)

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2314
Contract Title: Immunological Control of Reproduction
Contractor: Tulane University, Delta Regional Primate Research Center
Money Allocated: $23,936 (FY 70 Funds), $23,936 (FY 71 Funds)

Objectives: This is a contract to investigate: (1) the effects of testicular homogenate and sperm antigens on spermatogenesis in the male, and fertility in the female; (2) the effects of homogenates of fetal placental tissue on pregnancy maintenance or conception when given to gravid or nongravid females, respectively; (3) the effects of rabbit or sheep antiplacental sera on fertility in the female; (4) the characterization of the specific sperm or placental antigens involved in the above three sections. More specifically, during the first year, the applicants will immunize monkeys with autologous and homologous sperm, or testicular homogenates, and evaluate spermatogenesis, testicular reaction, and fertility. During the second contract period, they will repeat this in rabbits and monkeys using placental antigen. During the third phase of the program, they will attempt to identify the antigens and establish the degree of organ specificity of the antibodies formed against these antigens.

Major Findings: The work with the male that has been completed includes initial assessment of semen samples, hemicastrations for the purpose of obtaining the antigen, and injection of these animals with either complete or incomplete Freunds adjuvant with or without the antigen. Histology prior to injections indicates normal spermatogenic activity. Attempts to obtain reliable semen samples by electroejaculation have not been successful. It is proposed that the remaining testicle be biopsied at monthly intervals to assess the effect of immunization. The fiber of antibodies that will result from immunization will be ascertained by the hemagglutination technique.

Significance to Biomedical Research and Program of the Institute: The immunological studies undertaken in this project are directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2319
Contract Title: Development of a Laboratory Screening Procedure for Inhibitors of Progesterone Synthesis
Contractor: Harvard University
Money Allocated: $45,120 (FY 70 Funds), $45,500 (FY 71 Funds)

Objectives: The objectives of this contract is to define and evaluate agents which interfere with the enzymatic synthesis of progesterone in the corpus luteum. Specifically, the contract concerns the possiblity of finding substances which are specific inhibitors of the mitochondrial system which converts cholesterol to pregnenolone-specifically substances which act at the cytochrome P-450 site. It is suggested that substance exist which act specifically at this site and are non-toxic; such substances would be potential antifertility agents.

The immediate objective of this contract is to devise a convenient and relatively rapid screening procedure which will enable the prediction and preparation of substances which would be biologically effective for the termination of early pregnancy.

Major Findings: The biological aspect of the proposal dealt with direct screening of substances that would inhibit cytochrome P-450. This enzyme is the terminal link of the electron transport chain involved in the side-chain cleavage of cholesterol. The investigators have prepared this enzyme from corpora lutea in a soluble form. In the assay system it is crucial to know the amount of enzyme that is present, for otherwise the testing of inhibitors is meaningless. Different classes of inhibitors that have been studied include: (1) steroids, (2) barbiturates, (3) aniline derivatives, (4)glutethimide derivatives, (5) pyridines and (6) imidazoles. Using the adrenal 11β-hydroxylating P-450 system as the model system they have worked out structure activity relationships for these groups of compounds.

The next step will be to correlate the spectral screening with an *in vitro* bioassay. They can now predict which substances may be active on the basis of certain molecular groups and modecular weight to cite two characteristics. It is likely that the inhibitors act by attachment to the enzyme in a way which prevents reduction of the cytochrome P-450.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support development of laboratory screening procedures.

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2319 (page 2)

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Quantification of Prostaglandins
Contractor: The Upjohn Company
Money Allocated: $42,913 (FY 71 Funds)

Objectives: The objective of this contract is to develop a radioimmunoassay to measure prostaglandins and certain of their biologically active analogs, and subsequently to quantify these compounds in some body fluids and tissues.

Initially, prostaglandin E_2 and F_2 will be conjugated to bovine serum albumin and polylysine, then injected as a suspension with Freund's complete adjuvant into sheep, rabbits and guinea pigs to produce antibodies to the prostaglandin molecules. The affinity and specificity of the antibody will be characterized by comparing the competitive replacement of radiolabeled prostaglandin by several structurally related prostaglandin analogs. Conditions of incubation, methods of separation, and other alterable variables will be examined to optimize the sensitivity and precision of the assay. In addition, a limited number of prostaglandin analogs with biological activity that indicates a potential clinical advantage over natural compounds will be utilized similarly for assay development.

Circulating levels of prostaglandins will be quantitated during parturition in humans and at various stages of the menstrual cycle in monkeys. Absorption of prostaglandin from various formulations will be followed by quantitating prostaglandin in peripheral serum by radioimmunoassay. In vivo biological effects of administering antisera to prostaglandins will be determined

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on radioimmunoassays of prostaglandins.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: A Model for Studying Inhibition of LH Action on the Corpus Luteum
Contractor: Mayo Clinic and Mayo Foundation
Money Allocated: $36,924 (FY 71 Funds)

Objectives: A model system has been developed for studying the interaction of human luteinizing hormone (LH) with cells of the corpus luteum using slices of pseudopregnant rat ovaries. The object of this contract is to further validate this model, to define the structural requirements of the LH molecule, to define the requirements of the luteal cell for this interaction, and to seek inhibitors of this interaction. If such inhibitors are found, they may be highly specific abortifacients or contraceptives.

Effort will be made to relate the interaction of LH and the luteal cell to the steroidogenic action of LH. This will be done by assessing the stimulation of estrogen and progesterone synthesis using specific radioimmunoassays. The applicability of this model to human corpus luteum tissue and to the Leydig cells of the male will be investigated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research in the relationship between LH and the function of the corpus luteum.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Development of an Automated Immunoradiomimetric Assay System
Contractor: Veterans Administration Hospital, Palo Alto, California
Money Allocated: $59,110 (FY 71 Funds)

Objectives: The objective of this contract is to establish a simple, automated immunoradiometric assay device, capable of measuring low concentrations of a wide variety of antigens. The assay device is made possible by the prior development (by the applicant) of a new type of immunological assay system (the immunoradiometric assay). In this method, purified radioactive antibodies are used as reagents to convert all the unknown antigen into a directly detectable complex. Unreacted antibody is removed by reaction with an insoluble antigen immunoadsorbent. An important but parallel part of the project is the investigation and development of alternative antibody labels.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the development of new types of immunological assay systems.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Effect of Prostaglandins on Ovarian Function
Contractor: University of Miami
Money Allocated: $52,491 (FY 71 Funds)

Objectives: The objective of this research is to study the in vivo effect of prostaglandin $F_{2\alpha}$ on the corpus luteum of the menstrual cycle in women in order to determine what acute effects this compound has on the corpus luteum and to determine if it can be used as a luteolytic agent in humans. The volunteer subjects will be divided into two groups. The first group of normal healthy ovulating non-pregnant females will receive an infusion of prostaglandin $F_{2\alpha}$ at different times in their secretory phase of the cycle. This will be considered the control group. The parameters for corpus luteum function will be the measurement of plasma progesterone in the peripheral venous blood. The second group will consist of patients already scheduled to undergo abdominal gynecologic surgery for non-endocrine disease. Their surgery will be scheduled at a time a corpus luteum of a certain age can be expected. Prostaglandin $F_{2\alpha}$ administration will begin at varied times before surgery and progesterone will be measured in peripheral venous blood and in blood obtained from both ovarian veins. In addition, steroidogenesis of incubated luteal tissue will be measured in vitro.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the effects of prostaglandins on the corpus luteum.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2093
Contract Title: Study of Polyamines and Ornithine Decarboxylase as a Potential Contraceptive Measure
Contractor: Worcester Foundation for Experimental Biology
Money Allocated: $30,143 (FY 69 Funds), $33,740 (FY 70 Funds), $16,869 (FY 71 Funds)

Objectives: The overall purpose of this program is to establish the primary factors which regulate the metabolism of the polyamines in the reproductive tract, to investigate the role of these substances in the development and growth of the reproductive tract and to determine whether impairment of ornithine decarboxylase activity is a viable means of preventing ovulation.

Major Findings: In screening the tissues for decarboxylase activity, it was found that the ornithine decarboxylase activity of the ovaries showed a sharp rise and decline on the evening of proestrus. Subsequently, it was observed that this stimulation appeared to be under LH control. This enzyme has now been reported in the chick oviduct, rat uterus, prostate and ovary and, in every instance, appears to be under hormonal control.

In the pregnant rat, the investigator observed:

1. Ornithine decarboxylase develops steadily in the fetal placenta until term.

2. At term, 90 percent of the ornithine decarboxylase activity of whole placenta is contained in the fetal placenta.

3. At term, 75 percent of diamine oxidase activity of whole placenta is found in the maternal side.

4. Histidine decarboxylase activity of the placenta is low throughout pregnancy. These observations provide strong evidence that the substrate for high diamine oxidase activity found during pregnancy is, in fact, putrescine.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2094
Contract Title: Ultrastructural and Cytochemical Studies of Trophoblast Development and Implantation in the Rabbit
Contractor: University of Houston
Money Allocated: $18,829 (FY 69 Funds), $29,571 (FY 70 Funds), $29,571 (FY 71 Funds)

Objectives: The main objective of this research is to study, at the ultrastructural level, the early stages of development and implantation in the rabbit. Although the work is primarily descriptive, some cytochemical methods are being employed to evaluate the role of lysosomes in implantation and in the invasion of the maternal tissues by the syncytial trophoblast.

Major Findings: Results indicate that the trophoblast is composed of at least three distinct cell types. The pyramidal cells of the four day embryo found at the periphery of the polar cap are closely associated with the inner cell mass. The investigator believes that the newly differentiated trophoblast cells arise from the embryonic mass itself. The attenuated trophoblast cells found along the lateral sides of the blastocyst and the cells found at the abembryonic pole are also described.

In the seven and eight day embryo, there are three distinct cell types in the trophoblast; all play an active role in implantation.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the process of implantation and investigate ways in which it may be altered for the purpose of fertility regulation.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2126
Contract Title: The Physiology of Ovulation
Contractor: Trinity University
Money Allocated: $50,005 (FY 69 Funds), $51,493 (FY 70 Funds), $18,526 (FY 71 Funds)

Objectives: The objective of this continuing contract research is to study in mammals the processes related to the phenomenon of ovulation. Although a number of techniques are being employed in these studies, electron microscopy is being emphasized in an ultrastructural examination of ovarian changes preceding and during ovulation.

Major Findings: A variety of chemicals which could be involved in the disruption of follicular connective tissue during ovulation were incubated with strips of sow follicles to determine their effect on the tensile strength of the follicle wall. Commercial preparations of collagenase, elastase, protease, trypsin and chymotrypsin caused significant reduction in the tensile strength of sow follicles in vitro. In addition a series of non-enzymic agents were tested and ascorbate and diketogulonate were shown to reduce tensile strength.

Sow follicles were assayed for general proteolytic activity at several intervals from ovulation. The only enzyme present in measurable quantity was cathepsin; activity did not change significantly as ovulation neared.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to support research devoted to elucidating the mechanisms responsible for ovulation and potential means of altering these mechanisms for the purpose of fertility regulation.

Proposed Course: Termination in 6 months.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2127
Contract Title: Characterization and Environmental Control of Mammalian Oocyte Maturation
Contractor: University of Nevada
Money Allocated: $20,617 (FY 69 Funds), $24,323 (FY 70 Funds), $12,162 (FY 71 Funds)

Objectives: When mammalian oocytes are removed from the Graafian follicle, they undergo meiotic maturation independently of hormonal control. The applicant previously has shown that if the ovum is cultured within isolated follicles it remains in the arrested dictyate stage.

Based on these studies, this research is devoted to investigating the effects of various hormones on meiotic maturation. Follicles from human and cow ovaries are being used in studies to:

(1) determine the nature and timing of the stages of maturation in the human oocyte;

(2) determine the relationship between the oocyte and its surrounding follicle cells during oocyte and follicle maturation;

(3) determine the environmental conditions and changes which initiate and sustain the various steps of oocyte maturation;

(4) determine the fertilizability of oocytes matured under the controlled environment.

Major Findings: The investigator has dissected human follicles from ovaries and cultured them from 42-45 hours. In almost every case, the follicle cells remained healthy and showed signs of mitosis. The human follicle acts similarly to other mammalian species in inhibiting oocyte maturation.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the factors responsible for the initiation and maintenance of oocyte maturation.

Proposed Course: Termination in 6 months

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2128
Contract Title: Mechanism of Ovulation
Contractor: University of Miami
Money Allocated: $22,351 (FY 69 Funds), $23,600 (FY 70 Funds)

Objectives: The purpose of this contract is to investigate the mechanism of ovulation in various mammalian species. Particular attention is being paid to the function of cyclic AMP in this mechanism, and to possible ways in which cyclic AMP metabolism may be interrupted for the purposes of fertility control.

Major Findings: The investigators have explored the effects of intrafollicular injections of cyclic AMP and dibutyryl cyclic AMP on the process of lutenization and have concluded that cyclic AMP does not play a mediatory role in this process. However, it was demonstrated that cyclic AMP or dibutyryl cyclic AMP in addition to 1 ug of LH did reduce the percentage of lutenization of the injected follicles. Neither 5'-AMP nor 3'-AMP had an inhibitory effect, establishing to a certain degree the specificity of the cyclic AMP inhibition of lutenization.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to develop non-steroidal methods for contraception through inhibition of ovulation.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2129
Contract Title: Meiosis and Fertilization of Human Ova In Vitro
Contractor: Albert Einstein College of Medicine
Money Allocated: $37,301 (FY 69 Funds), $38,918 (FY 70 Funds)

Objectives: This research is designed to: 1) improve methods for the recovery of ova from women and for their maintenance in vitro; 2) determine the time sequence of the meiotic events of the ovum after its removal from the ovarian follicles; 3) study chromosome morphology and behavior during female meiosis; 4) establish methods for fertilizing rabbit and human eggs in vitro and study the cleavage stages.

Major Findings: Improved methods of culturing human ova have been established. Considerable number of ova have been recovered from untreated women, women taking oral contraceptives and women using contraceptive devices. Present studies are being conducted to establish the time sequence of meiotic events and to ascertain chromosome morphology and behavior.

Significance to Biomedical Research and Program of the Institute: These studies are relevant to the general area of contraception since the production of fertilized human ova in vitro appears the only way to study the physiology and pharmacology of the preimplantation stages.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2130
Contract Title: Role of Cyclic AMP in the Actions of Estrogen, Progesterone and Relaxin on Uterine Metabolism
Contractor: Emory University
Money Allocated: $24,427 (FY 69 Funds), $27,700 (FY 70 Funds), $14,487 (FY 71 Funds)

Objectives: This investigation is concerned with the relationship of estrogen, relaxin, and progesterone, alone or in combination with cyclic AMP, and the role of the latter, in the regulation of uterine metabolism during various phases of the ovarian cycle.

Major Findings: Uterine phosphorylase activity has been measured after treatment with various hormones and during the estrus cycle. Results indicate that treatment of ovariectomized rats with estrogen increases phosphorylase activity in the resting uterus and that phosphorylase activity in the resting, unstimulated rat uterus increases during the estrus cycle at those times when estrogen secretion by the ovary is known to be increased. Progesterone tends to reverse the effects of estrogen.

Significance to Biomedical Research and Program of the Institute: The metabolic studies undertaken in this project are directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Termination in 6 months.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2141
Contract Title: Blastocyst Expansion and Pre-ovulatory Follicle Swelling
Contractor: University of Pennsylvania School of Veterinary Medicine
Money Allocated: $23,928 (FY 69 Funds), $28,697 (FY 70 Funds), $44,539 (FY 71 Funds)

Objectives: The objective of this research project is to investigate a much neglected area of reproductive physiology: the mechanisms providing for the accumulation of fluid inside the expanding blastocyst and within the pre-ovulatory follicle. The objective ultimately is to find ways to influence these phenonema as a means toward fertility regulation. The specific projects undertaken are aimed at determining whether or not active transport of sodium, potassium or chloride ions occurs during these rapid periods of growth. This is being done by determining transmembrane potential; ionic flux studies using radioactive calcium, sodium, and chloride; the determination of short circuit current and its correlation with ionic flux; and finally the influence of hormones and drugs on the blastocyst and follicle expansion and in vivo trials with promising compounds. All work is done either in vivo or in vitro with the rabbit.

Major Findings: Data indicate that the preimplantation rabbit blastocyst maintains a negative potential difference (inside negative) across the trophoblast wall. This potential difference is dependent on the external Na^+ and Cl^- ions and the blastocyst must utilize metabolic processes to maintain this potential difference.

A successful method has been developed for the continuous-flow perfusion of the six-day rabbit blastocyst; the electrical properties across the epithelial trophoblast of this preparation have been determined. The perfusion technique consists of flushing blastocysts from the uteri of naturally mated New Zealand rabbits, securing one embryo in a specially designed environmental chamber, and inserting glass perfusion pipettes into opposite sides of the blastocyst. The trophoblast epithelium adheres naturally to the glass pipettes forming a tight seal.

Estrogen, progesterone, pitressin, epinephrine, FSH and LH were screened for their effects on the transtrophoblast potential difference. There was a decrease in the potential difference following exposure to progesterone but no effect with the other hormones. These studies are only preliminary but could serve as a basis for selecting hormones and other agents for a more detailed investigation of their effects on the measured electrial parameters.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Contract Number: 69-2141 (Page 2)

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2202
Contract Title: The Role of Cortical Granules in Fertilization
Contractor: Mount Sinai School of Medicine
Money Allocated: $59,389 (FY 69 Funds), $44,532 (FY 70 Funds), $36,913 (FY 71 Funds)

Objectives: Isolated cortical granules from homogenates of sea urchin, fish, and frog eggs, are being characterized chemically; and their behavior in the presence of acrosomal extracts and agents that induce parthogenetic development assessed. The initial emphasis on echinoderm eggs was dictated by the necessity of having large numbers of eggs for differential centrifugation. The studies will be extended to mammalian eggs using histochemical methods in place of isolation and chemical analysis.

Major Findings: Intact cortical granules have been isolated from homogenates of unfertilized sea urchin eggs and purified approximately 25 fold. The granules were shown to be rich in acid phosphatase activity. In addition, the enzyme B-1,3-glucanase was shown to be associated with the cortical granule compartment in the unfertilized sea urchin eggs. Half of the glucanase activity in the eggs is secreted into surrounding sea water during the first three minutes after fertilization which corresponds directly to the time when the cortical reaction is taking place.

Cytochemical studies of smear preparations indicated that almost all the particles visible in the cortical granule fractions stained metachromatically or contained sulfated acid mucopolysaccharides. Results indicate that sulfated acid mucopolysaccharides in the cortical granules of the egg play an essential role in fertilization and in establishing the block to polyspermy. These sites have been identified as potentially useful targets for antifertility effects of quaternary ammonium drugs. Pilot experiments designed to test this hypothesis are currently in progress using rats. The data suggest that CTMAB (cetyltrimethyl-ammonium bromide), CPC (cetylpyridinium choloride), and TEAC (tetraethylammonium chloride) were effective in reducing fertility.

Significance to Biomedical Research and Program of the Institute: Little is known about the composition and enzymatic properties of cortical granules and as a fundamental study the proposed work has real scientific merit. It is also related to the contraceptive development program. The true significance of these granules in mammalian eggs is not yet known.

Proposed Course: Termination in 6 months.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2210
Contract Title: Study of Uterine Cytoplasmic Protein Synthesis Throughout the Estrus Cycle and the Effect of Estrogens and Progestins on These Systems
Contractor: Detroit Institute of Cancer Research
Money Allocated: $82,947 (FY 69 Funds), for two years; June '69 to June '71

Objectives: The investigator is examining several components of the protein synthesizing system in the uterus, by comparing unstimulated with stimulated endometrium, isolating amino acid transferases and ribosomes, and by correlating amino acid uptake with transfer RNA and protein formation. The *in vitro* effects of steroids and other contraceptive agents will then be evaluated in this system.

Major Findings: The pH 5.2 enzymes have been isolated from immature, estrus and diestrus porcine uteri. This system was shown to have similar capacity to form aminoacyl-tRNA after isolation from both estrus and diestrus porcine uteri. The effect of addition of various estrogens to these systems was studied.

Microsomes isolated from porcine uteri were able to synthesize protein from aminoacyl-tRNA. The rate of synthesis was greatly stimulated in those microsomes isolated from mature uteri. Data from experiments with mature or immature components have been analyzed to provide the conditions for maximum protein synthesis. Both systems responded to the addition of artificial messenger RNA (poly U).

Significance to Biomedical Research and Program of the Institute: Studies directed towards understanding the mechanisms of protein synthesis in the uterus are directly related to the purposes of the program.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2061
Contract Title: Control of Ovulation and Capacitation in Non-Human Primate
Contractor: Michigan State U.
Money Allocated: $24,351 (FY 69 Funds), $18,100 (FY 70 Funds), $26,314 (FY 71 Funds)

Objectives: The objectives of this research project are: 1) to develop a reproducible technique for induction of ovulation in primates; 2) to determine the fertilizable life of primate ova; and 3) to carry out further studies on the need for sperm capacitation in the primate.

Major Findings: The basic cycle characteristics of the Cynomologous macaque colony have been established. The mean cycle length is 29.4 days with a duration of menstrual flow of 3.6 days. Using laparascopy and controlled mating period the investigator is establishing maxima for reproductive efficiency in both male and female monkeys. Through the use of laparascopy and photography they are establishing the follicle ovulatory patterns in these macaques. Rapid follicular growth does not occur until 18 to 20 hours prior to ovulation.

In the squirrel monkeys megesterol acetate at 500ug/day blocks induced ovulation.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies on the necessity for capacitation in primates.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2150
Contract Title: Mammalian Oocyte Maturation and Fertilization In Vitro
Contractor: University of Pennsylvania
Money Allocated: $39,945 (FY 70 Funds), $41,533 (FY 71 Funds)

Objectives: The objective of this research project is to develop an in vitro system for maturation, fertilization, cleavage, and development of the fertilized mouse egg to the blastocyst stage; such blastocysts will then be transplanted into a foster mother to obtain living young. In vitro culture of mouse ova from the 1-cell stage to the blastocyst has already been achieved, but considerable work remains to determine the role of the follicle cell in maturation, the possible dependence of oocyte maturation on hormones, and reproducible conditions for in vitro fertilization. These aspects of the problem will be attacked systematically by culture of oocytes within intact follicles, addition of follicle cells to medium of isolated ova, and addition of amino acids, cofactors, hormones, etc. to the medium.

Major Findings: Oocytes can be matured in vitro, fertilized in vitro and then develop into viable fetuses when transferred to recipient mothers. Both fertilization rate and the percentage of developing fetuses (33.0% and 3.2% respectively) are considerably lower than when maturation of oocytes is accomplished in vivo (90.1% and 23.6% respectively).

Study of nuclear and physiological maturation of oocytes grown in vitro is determined to a considerable degree by the complexity of the medium and whether or not the oocytes were surrounded by cumulus cells.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of culture techniques for the in vitro investigation of ovum-follicle interrelationship, and the hormonal dependence of ovum maturation and fertilization.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2151
Contract Title: Studies on the Antigenic Status of the Ovum
Contractor: Philadelphia College of Pharmacy and Science
Money Allocated: $24,730 (FY 70 Funds), $29,056 (FY 71 Funds)

Objectives: The objective of this research project is to obtain information on the development of antigens in the mouse, especially the strong histocompatibility-2 (H-2) antigen. The importance of the histocompatibility loci in adult mice, rats and man has been established. Relatively little, however, is known about these antigens during development. In these studies, the investigator will determine the time at which the H-2 antigen appears in pre-implantation mouse embryos, and the presence of "embryo-specific" and other transplantation antigens in early embryos. Two-cell mouse embryos will be grown in vitro and the effects of specific allo-antisera on embryonic and trophoblast cells studied during the first eight days after escape from the zona and attachment to the slide. By these methods and immuno-fluorescence techniques, the precise time of appearance and cell localization of antigens will be determined, as well as the effects of antisera on embryo development in vitro.

Major Findings: Studies completed so far indicate that it is not possible to detect H-2 antigens on the surface membrane of mouse blastocysts that (a) have had the zona pellucida removed enzymically or (b) have been cultivated in a relatively simple medium to the point where they "hatch" out from the zona pellucida, and begin to flatten out onto the glass. Although some sera known to contain both H-2 and non-H-2 antibody destroyed the eggs, those containing H-2 only failed to impede blastocyst formation even in the presence of guinea pig serum as a source of complement. These results suggest that if present, H-2 antigenic sites were either so sparsely distributed, or so spatially oriented, that a cytolytic effect was precluded.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the potentially unique antigenic status of the developing embryo.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2155
Contract Title: The Antigenicity and Immunology of the Trophoblast and the Potentials for the Use of Anti-Trophoblast Serum as a Contraceptive
Contractor: University of Michigan Medical Center
Monely Allocated: $38,229 (FY 70 Funds), $56,586 (FY 71 Funds)

Objectives: When mouse blastocysts are placed beneath the kidney capsule of irradiated hosts, a considerable proliferation of the trophoblast occurs. By combination of the organ culture grid method and the monolayer technique, an almost pure culture of trophoblast cells can be obtained. These cells can be harvested and injected into rabbits an anti-mouse trophoblast serum obtained. Injection of this serum into pregnant mice rapidly causes abortion at all stages, but liver necrosis and chronic nephritis develops in the females. However, if the antiserum is fractionated on a DEAE column, a fraction can be obtained which causes abortion, but does not affect the liver and kidney.

The objective of this research project is to determine the feasibility of applying this technique to the Rhesus monkey. Essentially these same techniques will be used to prepare Rhesus monkey trophoblast antigen and to produce rabbit anti-monkey trophoblast antibody. It will then be established that purified anti-trophoblast antibody is capable of producing abortion reliably in the monkey, and that re-mating of the monkey is possible without delayed cellside effects of this treatment on reproductive and non-reproductive tract tissues and organs will also be determined.

Major Findings: The investigator has successfully immunized rabbits and goats with placental cell preparations and prepared anti-monkey placental cell preparations and prepared anti-monkey placental globulin.

The anti-placental globulin from either rabbits or goats was administered IV to pregnant monkeys. The amount of globulin given was 450 to 650 mg. In 4 out of 5 cases abortion ensued 2 to 4 days after the last of 3 injections. The 5th animal experienced anaphylaxis on the 3rd day of injection. Animals injected with normal goat serum globulin did not abort. Subsequently one additional monkey was given goat anti-monkey placental extract, but did not abort despite a repeat series of injections up to a total of 13,000 mg of protein. In this case immunoelectrophoretic pattern of the antibody differed from the one obtained with the effective antibody. In two post-aborted monkeys no pathological effects in the liver, spleen and kidneys were observed. In all of the abortuses there was massive cell infiltration into the decidua and around the trophoblast, with occasional autolytic changes in the fetus.

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2155 (page 2)
Contract Title: The Antigenicity and Immunology of the Trophoblast and the Potentials for the Use of Anti-Trophoblast Serum as Contraceptive

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies devoted to developing new methods of interrupting pregnancy in its initial stages.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2161
Contract Title: Studies on the Acrosomal Enzymes Effecting Penetration of the Zona Pellucida
Contractor: University of Pennsylvania School of Medicine
Money Allocated: $28,250 (FY 70 Funds), $37,193 (FY 71 Funds)

Objectives: In previous work this investigator and his colleagues have identified a trypsin-like enzyme in the acrosome effecting penetration of the zona pellucida. In their system in vitro fertilization could be prevented by use of trypsin inhibitors. The objective of this project is to carry out additional research on the nature and number of enzymes present in the acrosomal complex. An effort will also be made to localize the trypsin-hyaluronidase complex to the inner membrane of the acrosomal cap employing ferritin-labeled antibodies and inhibitors together with electron microscopy. Finally, a search will be made for naturally occurring specific inhibitors of the zona-lysing enzymes in the belief that such inhibitors might be effective antifertility agents.

Major Findings: The trypsin-like enzyme extracted from sperm acrosomes has been given the name "acrozonase." This enzyme has been observed in subcellular fractions of acrosomes from rabbits, rhesus monkeys and men. This enzyme occurs together with hyaluronidase in a 59,000 molecular weight complex. Using florescein-labelled trypsin inhibitors and counterstaining, the localization of "acrozonase" in the acrosome has been confirmed. Epididymal, ejaculated and capacitated rabbit sperm could be stained by the inhibitor.

Subcellular fractionation of spermatozoa indicates that acrozonase activity is higher in the acrosome than sperm heads. The ratio of trypsin-like activity to hyaluronidase activity is essentially the same in rabbit acrosomes and sperm heads, but in the rhesus monkey acrosomes it appears to be higher than in sperm heads.

The investigator has observed no corona cell dispersing factor in the extracts of acrosomes. Its location may be outside of the acrosome, or the present extraction procedure inactivates it. The human cervical mucus antitrypsin does not inhibit the activity of either human or rabbit acrozonase. The synthetic inhibitor p-aminobenzamidine appears to be a fairly selective inhibitor of acrozonase (73% inhibition) as opposed to its effect on pancreatic trypsin (20%).

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies devoted to elucidating the sperm-associated mechanisms which play a part in fertilization of the ovum.

Contract Number: 70-2161 (Page 2)

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2163
Contract Title: In Vitro Fertilization of Primate Ova
Contractor: University of Pennsylvania
Money Allocated: $48,740 (FY 70 Funds), $55,777 (FY 71 Funds)

Objectives: The primary objective of this research project is to develop a reproducible procedure for in vitro maturation of human oocytes and fertilization of ova. The investigator has a successful procedure for in vitro fertilization of rabbit ova and will now extend this experience to the rhesus monkey and man. Ova will be recovered from the oviducts of monkeys and exposed to a suspension of washed sperm or sperm incubated in utero in mid-cycle monkeys. It is expected that these studies will soon indicate whether capacitation of sperm in the female tract is necessary in the primate.

It is also planned to repeat published observations on maturation of monkey and human ovarian oocytes and their fertilization in vitro. The potential for continued development of oocytes matured in vitro will be assessed by transferring them to oviducts of monkeys previously inseminated. They will be recovered later and examined for evidence of in vivo fertilization.

In parallel with these studies, investigations on defining more exactly the biochemical and physical conditions for in vitro fertilization in rabbits will be continued.

Major Findings: Three hundred forty one monkey follicular ova have been used in 24 attempts to accomplish in vitro fertilization. Only one monkey ovum cleaved after incubation with spermatozoa. Ovum maturation did take place whether the ova came from normal or gonadotropic stimulated ovaries. Experimental conditions that lead to successful cleavage of human follicular ova were of no avail. Three ova recovered from oviducts did not cleave either, when exposed to sperm that were incubated in utero.

Fifty human follicular ova were exposed to human spermatozoa that were incubated in monkey uteri. Among these 8 ova cleaved. When 15 ova were exposed to washed human spermatozoa, none of them cleaved. This suggests a need for capacitation in the human. Efforts can now be directed toward identification of early stages in the fertilization process and proof of involvement of the sperm cell in this process.

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2163 (page 2)
Contract Title: In Vitro Fertilization of Primate Ova
Contractor: University of Pennsylvania

Conditions for the invitro fertilization of rabbit ova have been imporved so that an almost 80% fertilization rate is achieved. A greater proportion of ova can usually be fertilized in vitro, when the ova are recovered from the ovarian surface rather than when the ova are recovered from the oviducts of the same ovum donors at the same time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies on the development of in vitro systems for the investigation of oocyte maturation and fertilization under controllable conditions.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives an is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2166
Contract Title: Uterine Secretions and Implantation
Contractor: The Johns Hopkins University
Money Allocated: $39,749 (FY 70 Funds)

Objectives: The objective of this contract is to investigate the factors which regulate blastocyst development and implantation. Using mice, attempts will be made to determine whether uterine secretions directly influence the blastocyst. Both biological and biochemical methods will be employed to characterize the active uterine substances. The proposed biologic tests, which are qualitative in nature, include both *in vivo* and *in vitro* approaches. In the former, attempts will be made to induce implantation in mice, during delayed implantation, by intraluminal administration of the appropriate uterine secretions. In the latter, blastocysts which have been grown from 1- or 2-celled stages in chemically defined media, and diapausing blastocysts which have been recovered from mice during delayed implantation, will be compared with respect to their ability to respond to various uterine secretions or sex hormones *in vitro*. Several parameters of biosynthetic activity will be tested.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the factors responsible for the successful implantation of blastocysts in the mammalian uterus.

Proposed Course: Termination. Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2252
Contract Title: The Mechanism of Follicular Rupture
Contractor: Columbia University College of Physicians and Surgeons
Money Allocated: $28,300 (FY 70 Funds), $14,150 (FY 71 Funds)

Objectives: The objective of this research project is to examine the mechanism of follicular rupture and to determine the feasibility of modifying this mechanism as a method of fertility control. Specifically, the investigator will attempt to isolate the enzyme responsible for rupture of the follicle, identify the enzyme, purify it, and prepare derivatives. By the use of these derivatives or inhibitors of the enzymes, the investigator will attempt to influence ovulation.

Major Findings: Experimental evidence obtained to date indicates that a 0.5% solution of pronase can be used for the isolation of follicles. Liquor folliculi exhibits collagenase activity. Follicles grown *in vitro* and then ovulated produce changes in the gells on which they are grown.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the mechanism of ovulation and its possible alteration for the purpose of fertility control.

Proposed Course: Termination in six months.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2254
Contract Title: Studies of the Estrogen-Binding Protein (Estrogen Receptor) in the Human Uterus and Anterior Pituitary and in the Rodent Uterus Anterior Pituitary and the Hypothalamus
Contractor: University of Rochester
Money Allocated: $33,450 (FY 70 Funds), $16,725 (FY 71 Funds)

Objectives: The objective of this proposal is to compare the molecular properties of the estrogen-binding protein of human uterus and anterior pituitary and of rat uterus, anterior pituitary, and hypothalaums to see whether the receptor substance of one tissue is similar to, or different from, that of another. The receptor proteins will be compared as to sedimentation characteristics, gel filtration chromatography, electrofocusing, binding constant, and the influence of anti-estrogenic compounds on the binding properties.

Major Findings: Numerous experimental procedures have been used, all of which indicate that the estrogen receptors of the rat anterior pituitary and uterus have identical molecular characteristics and estrogenic binding specificities. The sedimentation coefficient for the estrogen-binding macromolecule from both sources was 8S. Several estrogen antagonists reduced binding of E_2 to the same degree in the uterine and pituitary macromolecules. The binding kinetics are the same for the estrogen receptors from both sources. On basis of wet tissue weight uterus has 60% more binding capacity than does the anterior pituitary; on basis of DNA it becomes a 4 to 3 ratio. Through the use of different enzymes the investigator has shown that a protein moiety is necessary for binding activity, whereas lipid, phosphates or nucleic acids are either not present or not essential.

The properties of the human uterine receptor appear to be very similar to the ones observed for the rat.

The binding affinity of the uterine receptor for ethinyl estradiol is somewhat higher than for estradiol.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of steroid binding proteins in reproductive and central nervous tissues.

Proposed Course: Termination in six months.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2258
Contract Title: Steroid Hormone Binding in Reproductive Organs
Contractor: Yale University
Money Allocated: $39,141 (FY 70 Funds), $50,795 (FY 71 Funds)

Objectives: The objective of this research project is to study various aspects of the interaction of contraceptive drugs and potential contraceptive agents with steroid binding molecules in organs concerned with reproduction. The investigator will continue studies directed towards *in vitro* comparisons of estrogen binding molecules from the rat uterus, vagina, anterior pituitary, and hypothalamus. The affinity of estradiol for binding to macromolecules from the supernatant fraction of these organs will be determined after *in vitro* incubation, and various drugs will be examined for their ability to compete with tritiated estradiol for macromolecular binding. To facilitate these studies, the investigator will attempt to develop a gentle and rapid purification procedure for estrogen binding macromolecules utilizing as the separating medium, estradiol reversibly linked to a standard chromatographic resin.

Major Findings: The dissociation equilibrium constants for estradiol are approximately 2×10^{-10} for the supernatant fraction from the uterus, hypothalamus and anterior pituitary. Similar observations for ethinyl estradiol give K_i of about 1×10^{-10}M; for mestranol K_i was 5×10^{-8}M. These data indicate that the estrogenic components of oral contraceptives do not exhibit organ specificities, and that activity of mestranol is most likely due to conversion to ethinyl estradiol.

Work is progressing on the preparation of a new system for the isolation of estrogen binding macromolecules. The system entails the synthesis of an estradiol-resin complex which in turn binds the macromolecule.

The macromolecular complex prepared from human endometrium exhibits estrogen binding of the same order as does the one from the rat uterus. This also holds true for monkey endometrium.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the steroid binding mechanism in reproductive and central nervous system tissues.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2305
Contract Title: Study of Estrogen and Progesterone Binding Substances
Contractor: Mayo Foundation
Money Allocated: $40,960 (FY 70 Funds), $41,740 (FY 71 Funds)

Objectives: The investigator plans to develop a radioimmunoassay for estradiol, estrone, and progesterone and to measure the concentration of these hormones in arterial blood, in the uterus, and in the uterine secretions during the normal cycle and early pregnancy in the rat. Some observation may also be made in man and correlated with data obtained in the rat. In addition the investigator plans to develop a radioimmunoassay for the uterine estrogen receptor proteins; to purify and characterize the human cytoplasmic and nuclear estrogen receptor and study their interconvertibility; to study the structure requirements for binding between the receptor and the steroid; and to seek evidence for the existence of a uterine progesterone receptor protein.

Major Findings: The radioimmunoassay for estrogen (E_1+E_2) is presented. The method is simple and easy to perform; one technician can easily process 24 samples, in duplicate, in 2 days. Values are presented for normal males, normal females, postmenopausal females, hypogonadal females and children. The values quoted for normal males were 87.2 $\pm$ 25.9; for normal females 168.1 $\pm$ 103.1; postmenopausal females 52.0 $\pm$ 33.0; in hypogonadal men only 1 out of 9 values was above 30; hypogonadal women averaged about 30, and children (4 samples) were all below 30. These values are in pg/ml and represent mean $\pm$ SD.

An antibody against 11-hydroxyprogesterone antigen has been purified and it appears to be sensitive to progesterone.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on estrogen and progesterone binding substances.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2309
Contract Title: Transfer of Maternal Macromolecules to Mammalian Eggs
Contractor: University of California Medical School
Money Allocated: $29,000 (FY 70 Funds), $39,480 (FY 71 Funds)

Objectives: The principal investigator will examine in detail the macromolecular transfer process in the preimplantation mammalian embryo, working primarily with oocytes and embryos of the mouse and rabbit. There are four general areas of investigation: (1) the composition and origin of mouse and rabbit blastocyst fluid. The blastocyst fluid constituents will be analyzed and the evacuated blastocysts placed in various media. By this means it is hoped to learn more of the factors controlling the passage of macromolecules into blastocysts. Blastocysts will also be cultured in various antisera to see if they are detrimental to development. If such sera are found, females will be immunized passively or actively to see if development stops in vivo. (2) The composition and origin of rabbit oviductal fluid. Studies will be made of the macromolecular components of rabbit oviduct secretions and these will be compared with the constituents of blood of normal female rabbits. Antisera or analogs will be tested for adverse effects on embryonic development. (3) Electrophoretic studies will be conducted of the enzymes and antigens of the preimplantation mouse embryo. Attempts will be made to identify components which might be blocked to arrest development. (4) Attempts will be made to destroy selectively specific tertiary follicles in the mouse ovary by antifollicle sera.

Major Findings: 1. Culture of mouse morulae with normal rabbit serum (NRS) resulted in death of the morulae unless NRS was dialyzed for 5 days.
2. Mouse morulae cultured in BMOC with addition of either bovine plasma albumin, mouse serum or human serum albunia achieved the same stage of development in all media. Electrophoresis of washed blastocysts from the three different culture media showed quantitative differences in electrophoretically detectable protein which were highly reproducible. The meaning of this change is discussed.
3. Different metabolic inhibitors were added to the culture media containing mouse morulae. Dinitrophenol was lethal at concentration of 10^{-3} and 10^{-4} mg/ml of medium and at 10^{-5}, 87% of the morulae developed into blastocysts. Actinomycin D at 10^{-3}, 10^{-4} and 10^{-5} mg/ml was toxic to all morulae. With theophylline at 10^{-3}, 58% of morulae reached blastocyst stage. At 10^{-4}, the percentage was 70, and at 10^{-5} it was 89%.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on preimplantation mammalian embryos.

Contract Number: 70-2309 (Page 2)

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2318
Contract Title: Uterine Biogenic Amine and Estrogen Relationships, and The Effects of Intrauterine Contraceptive Devices.
Contractor: State University of Iowa
Money Allocated: $25,356 (FY 70 Funds), $37,492 (FY 71 Funds)

Objectives: The investigator plans to study the biogenic monamines norepinephrine, epinephrine, histamine and 5-hydroxytryptamine in the uterus under various physiological conditions. These include cyclic changes during the normal cycle, the effect of exogenous steroids, and the presence of an IUD. Fluorescence histochemistry of biogenic monamines will be used to study monamine localization in relation to (1) uterine blood flow in response to estrogen, (2) the ability of the fertilized ovum to implant, and (3) the effects of an IUD. Individual monamine levels will be manipulated selectively by administration of inhibitors of amine synthesis and the effect of such manipulation determined on uterine response to estrogen and ovum implantation.

Major Findings: The investigators could not substantiate the report that adrenergic nerve fluorescence was maximal at estrus and minimal at diestrus. They point out that norepinephrine content is not necessarily linearly-related to adrenergic nerve fluorescence.

Mast cell fluorescence in the uterus is considerably lower during diestrus, as compared to estrus. Fluorescence microscopy and electron microscopy of mast cells is highly correlated with the tissue histamine content.

Following administration of estradiol to ovariectomized rats adrenergic nerve fluorescence decreased in intensity around the arterioles near the mesenteric attachment. Mast cell fluorescence intensity, determined by microfluoremetric examination of individual cells, did not show any appreciable decrease at any time after estradiol. This study maybe complicated by the presence of 5-HTP in rat mast cells.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on metabolic effects of intrauterine contraceptive devices.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Endocrine Control of Human Oocyte Maturation
Contractor: Peter Bent Brigham Hospital
Money Allocated: $23,213 (FY 71 Funds)

Objectives: The objective of this contract is to explore a new approach to contraception involving the control of maturation of the human oocyte. Mammalian oocytes are in a resting stage of meiosis until shortly before ovulation. At this time meiosis resumes. The investigators wish to find out what hormones stimulate the resumption of meiosis in humans. They propose to administer these hormones before ovulation in order to induce a premature maturation of the oocyte. They anticipate that if this is done, when ovulation does occur the over mature oocyte will be incapable of being fertilized.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on new approaches to contraception involving the control of human oocyte maturation.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2097
Contract Title: Formation of Dihydrotestosterone in Male Sex Organs and Transport of Steroid Androgens from the Testis to the Epididymis
Contractor: University of Southern California
Money Allocated: $36,000 (FY 69 Funds), $45,250 (FY 70 Funds), $53,257 (FY 71 Funds)

Objectives: The contractor is studying epididymal relationships and formation of two androgens, testosterone and dihydrotestosterone. He is developing a method for submicrogram quantification of dihydrotestosterone in a biological sample in order to measure the levels of testosterone and dihydrotestosterone in testicular tissues, spermatic venous blood, spermatic lymph, corpus, and cauda epididymis.

Major Findings: The investigator has developed a method for the quantification of dihydrotestosterone in biological samples. The method uses an electron capture cell for steroid quantification following gas-phase chromotography and does not depend upon isotopes for end point of assay. In addition, a method has been perfected for simultaneous infusion of the left dog testis via the left spermatic atery and the left epididymis via the left epididymal artery. Results indicate that the mature dog testis secretes small amounts of dihydrotestosterone and that it is quite likely that non-Leydig cells convert testicular steroids to dihydrotestosterone. If radioactive ^{5}-pregnenolone is infused via the left spermatic artery, the true specific radioactivity of dihydrotestosterone in spermatic venous blood is not that of secreted testosterone. Thus, testosterone appears not to be the only precursor for dihydrotestosterone in the dog testis.

The epididymis was shown to contain several steroid metabolic enzymes and to secrete dihydrotestosterone into epididymal efferent blood. In addition, the investigator has demonstrated that the dog prostate secretes dihydrotestosterone into prostatic venous blood. He has been unable to show that ICSH or HCG influence this secretion.

Significance to Biomedical Research and Program of the Institute: This project is directly relevant to the contraceptive program since epididymal physiology has been identified as a particularly important topic for concentrated attention.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2099
Contract Title: Prostaglandins and Smooth Muscle Responses of Male Sex Organs
Contractor: University of Oregon
Money Allocated: $32,750 (FY 69 Funds), $34,744 (FY 70 Funds), $35,870 (FY 71 Funds)

Objectives: The objective of the project is to compare the effect of the prostaglandins E and F compounds on: 1) the smooth muscle responses in male sex organs (seminal vesicle, vasa deferentia, ductus epididymis seminiferous tubule); 2) the in vitro and in vivo smooth muscle response of the same organs to other smooth muscle stimulants such as adrenalin, serotonin, vasopressin, oxytocin and the neurotransmitters noradrenalin and acetylcholine; 3) the smooth muscle response in male sex organs of normal or castrated animals. The investigation is aimed at the understanding of the sites and mechanism of action of the prostaglandins in smooth muscles.

Major Findings: During the first contract period, the investigator tested the effects of prostaglandins on smooth muscle responses of male accessory sex organs to stimulating agents such as adrenalin, noradreralin, acetylcholine, serotonin, oxytocin, etc. The work was then extended, in the second contract year, to study the smooth muscle responses of the vas deferens to prostaglandins and neurotransmitter agents in the presence of various blocking agents such as atropine, phentolamine, propranolol, dibenzyline, and others.

Of the prostaglandins tested, PGE_2 provided the best potentiation of vas deferens responses to smooth muscle exicitants. In vitro and in vivo treatments of hamster vasa deferentia with the anti-fertility compound U-5897 were not effective in altering the smooth muscle responses of this organ to prostaglandins and the several agonists.

Following vasectomy, there was no significant differences in responses to prostaglandins. In addition, organs from castrated animals showed no pronounced differences in their muscle responses to PG's and the excitants.

Preliminary results from current experiments indicate the appearance of rhythmic contractility in estradiol treated castrate vasa and that PGE_2 exerts effective and prolonged potentiation of responses to neurotransmitter agents. Studies are also in progress incubating vasa deferentia in tritiated PGE_2 to enable localization of this compound by autoradiographic EM.

Significance to Biomedical Research and Program of the Institute: This project is related to the purpose of the contraceptive development program particularly in its effort to delineate the mechanism of sperm transport in the epididymis.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2099
Contract Title: Prostaglandins and Smooth Muscle Responses of Male Sex Organs
Contractor: University of Oregon

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2101
Contract Title: Formation of the Subacrosomal Mass (Perforatorium) of the Golden Hamster Spermatozoa
Contractor: Tulane University
Money Allocated: $14,451 (FY 69 Funds), $15,347 (FY 70 Funds), $15,347 (FY 71 Funds)

Objectives: The investigator is studying the origin of the perforatorium of golden hamsters' sperm by following initially the formation of the perforatorium in early spermatids by electron microscopy. The hamster is chosen because of the large size of the hamster perforatorium; however, these studies will be extended to primate spermatids later in the project. Histochemical studies are being performed, and chemical characterization of the perforatorium attempted.

Major Findings: The objective of this research is to study the mammalian sperm organelle known as the "perforatorium" which may play a crucial role in the early events of fertilization. Initially, the investigator examined the ultrastructure of the mature sperm head of hamsters and determined that the "perforatorium" was a lateral extension of the subacrosomal space and its contents. The substance of the "perforatorium" was found to be of two sorts, as evidenced by differential staining. A more dense substance was traced as the homologue to the so-called "apical body" of rabbit sperm; a less dense substance was shown to extend back as a thickened band of material over two flattened surfaces of the nucleus. Evidence was presented to show that "perforatorium" is exposed as the anterior most element of the spermatozoon prior to sperm penetration of the zona pellucida.

Sufficient observations have been accumulated to present an account of the development of the "perforatorium" in hamster spermatids and demonstrate homologies in sperm of primates, including those of the chimpanzee. The basic elements which comprise the subacrosomal complex or "perforatorium" of rodent sperm and the morphogenetic mechanisms which produce the subacrosomal complex are common to all mammalian species.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to support research on potential areas of male contraception.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2102
Contract Title: Metabolism in Spermatogenesis and Sperm Maturation
Contractor: Boston University
Money Allocated: $11,749 (FY 69 Funds), $14,770 (FY 70 Funds), $22,491 (FY 71 Funds)

Objectives: This research project is involved with determining the biosynthetic potential of testicular spermatozoa and of spermatozoa from the caput and cauda of the epididymis by studying the biosynthesis of certain key lipids. It may then be possible to uncover a critical stage of metabolism in sperm maturation which may then be vulnerable to specific inhibitors.

Major Findings: The investigator has made a survey of changes in biosynthetic potential of mammalian spermatozoa during their passage through the epididymis and demonstrated that spermatozoa passing from caput to cauda of the epididymis of the rat have the following changes in metabolic activity: 1) a decreased uptake of uridine -5-H^3, 2) an apparent increase in the uptake of amino acids, 3) a decline in the rate of biosynthesis of the lipids.

Non-flagettate cells (mainly spermatocytes) were isolated by filtration and centrifugation. Comparison of spermatocytes and epididymal spermatozoa revealed that the spermatocytes take up amino acid (C^{14}-leucine) into hot TCA-insoluble material and that this uptake is sensitive to inhibitors of protein synthesis whereas spermatozoa showed only a small uptake of leucine which was insensitive to these inhibitors.

Measurement of the rates of incorporation of C^{14}-glucose into the lipids of isolated epididymal spermatozoa showed a decline of activity in the spermatozoa from the cauda. Preliminary studies found that lipid metabolism may be sensitive to hormone action. Chlormadinone and cyproterone treatment appears to result in an increase in triglyceride labeling whereas testosterone, medroxyprogesterone and a non-steroidal drug (WIN 17,578) caused a decrease.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies directed towards the development of new, male methods of contraception.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2103
Contract Title: Biochemical Requirements for Fertilization and Development of Rabbit and Human Ova
Contractor: University of Georgia
Money Allocated: $34,122 (FY 69 Funds), $45,351 (FY 70 Funds), $46,519 (FY 71 Funds)

Objectives: This research project involves the study of at least three enzymes present in sperm, the corona-removing enzyme (CRE) and two trypsin-like acrosomal enzymes which, the applicant hypothesizes, are involved in the penetration and removal of the corona radiata, the zona pellucida and the vitelline membrane. The applicant further postulates that "decapacitation factor" (DF) is a low molecular weight material which inhibits the CRE and thus blocks fertilization or capacitation. Both rabbit and human materials are used to study these points. Included as a related objective is the development of an effective medium and the proper physical conditions for *in vitro* fertilization and cleavage of human ova obtained from ovarian tissue removed at local hospitals.

Major Findings: The objective of this research is to define the biochemical steps spermatozoa must carry out to penetrate the ovum. The investigator has demonstrated that the zona pellucida of the rabbit ovum is altered by sperm neuraminidase. The zona pellucida becomes more resistant to dissolution by a proteolytic enzyme (acrosin) of the sperm. Disc electrophoresis data suggests that the tertiary and quaternary structure of the zona pellucida is altered by the neuraminidase. The possible significance of a neuraminidase - sialic acid interaction in the adherence of spermatozoa to the zona pellucida is under investigation.

The purification of decapacitation factor (DF) is in the final stages. It is made up of three peptides with antifertility activity. Acrosin, a trypsin-like enzyme of sperm, has been purified 250 fold. It has a molecular weight of 55,000 and exists as a dimer. Isolation and purification of corona penetrating enzyme is now underway.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to elucidate the mechanisms involved in sperm capacitation and in the critical process of fertilization.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2104
Contract Title: Fine Structure and Function of the Accessory Glands of the Male Reproductive Tract
Contractor: University of Colorado
Money Allocated: $14,985 (FY 69 Funds), $12,750 (FY 70 Funds), $18,266 (FY 71 Funds)

Objectives: This study of the fine structure and function of the male accessory organs is being carried out principally in rats, with particualr attention being paid to: (1) effects of vasectomy, both proximal and distal, to the point of ligation of the vas; and (2) changes which take place in the Wolffian ducts of rat fetuses, before, during, and after onset of androgen secretion (at daily intervals from day 14 until birth). Electron microscopy and electron microscope radioautography are being used to follow the fate of radiolabeled precursor substances in a study of the secretory activity of the accessory glands.

Major Findings: The investigators have demonstrated that the cells of the rat urogenital sinus and Wolffian duct have a simple undifferentiated appearance prior to the onset of androgen secretion at day 15 of gestation. Changes in ultrastructure during fetal development are minor compared with post natal changes, but those that do occur appear to be correlated with the onset of androgen secretion by the fetal testes. "Intracytoplasmic confronting cisternae" of the granular endoplasmic reticulum become numerous in the urogenital sinus and Wolffian duct. Dilation of portions of the granular reticulum and the formation of many hemidesmosomes occur in the urogenital sinus. Few fine structural changes accompany formation of the seminal vesicles and the prostate. Rapid differentiation of the cells of the prostate begins during the second week after birth. The major changes include proliferation of the granular endoplasmic reticulum, growth of the Golgi apparatus, and the appearance of secretory vacuoles. The rat prostate appears to be capable of secretion as early as 10 days after birth. Large supranuclear lysosomes appear simultaneously with the initiation of growth of the secretory apparatus, suggesting that they may play a role in normal prostate function. The mature appearance of the prostate is attained by about 4-5 weeks after birth.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2105
Contract Title: Inhibition of Capacitation: A Possible Contraceptive Method
Contractor: University of Virginia Medical School
Money Allocated: $21,788 (FY 69 Funds), $22,956 (FY 70 Funds)

Objectives: This is a study of the effects of alterations in the hormonal state of the female reproductive tract on capacitation of sperm. The investigator is studying the effects of progesterone, antiestrogens, testosterone derivatives and other compounds on the capacitation of rabbit spermatozoa.

Major Findings: Capacitation of sperm in the rabbit oviduct can be interferred with, but not abolished, by progestational agents and antiestrogens. Capacitation of sperm in the uterus can be prevented by progestational agents. Treatment of female rabbits with norethindrone resulted in production of ova with lowered fertilization rate.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the phenomenon of capacitation and on potential means of inhibiting this phenomenon by hormonal manipulation of the female reproductive tract.

Proposed Course: Renewal not requested

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2106
Contract Title: Mammalian Sperm Motility and Ultrastructure
Contractor: Washington University
Money Allocated: $42,699 (FY 69 Funds), $40,543 (FY 70 Funds), $52,589 (FY 71 Funds)

Objectives: The objectives of this research project are: 1) to study in a variety of species both sperm ultrastructure and sperm motility in an attempt to correlate them; and 2) to study the fine structural differences in spermatozoa before and after capacitation.

Major Findings: Results obtained indicate that living spermatozoa have more than one type of motility pattern; frequently sperm swim in circular paths. The diameter of the circles which swimming spermatozoa describe varies greatly among species and between spermatozoa in the same sample.

Spermatozoa which swim in circles have what might be considered as an effective and a recovery stroke. The mid-piece bends sharply to one side and then straightens out rather than waving symetrically in both directions. These asymmetrical effective and recovery strokes may result in the circular paths which mammalian spermatozoa sometimes traverse. Sperm cells can and often do compensate for the circular tendency by flipping 180^{o} every few strokes or alternate strokes. Some of the films indicate that mammalian spermatozoa have another motility pattern on surfaces which looks like crawling. This will be further investigated by observing sperm motility on substrates such as monolayer tissue culture cells.

Spermatozoa of some species are extremely stiff whereas spermatozoa of other are flexible. The stiffness of the flagellar beat correlates positively with the cross sectional area of the dense fibers. These fibers, therefore, may serve as stiff elastic elements rather than contractile structures.

Studies on the ultrastructure of sperm heads have indicated very percise and hexagonally spaced periodic densities of center to center spacing of 40Å in the basal belt region of the acrosome. In many species, the sperm head is sticky and adheres to the slide. The point of attachment corresponds to the arc where minute vesicles and tubules are found. It is suggested that minute vesicles and tubules which coat certain areas of sperm heads may be involved in adhesion to objects and perhaps in attachment in fertilization. The investigator reports no convincing differences between "capacitated" and "non-capacitated" spermatozoa of the rat.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of

Contract Number: 69-2106 (page 2)
Contract Title: Mammalian Sperm Motility and Ultrastructure

the Contraceptive Development Branch to support research on the correlation of sperm function and ultrastructure especially as it is affected by capacitation.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2107
Contract Title: Morphological and Experimental Studies on Spermatogenesis, Sperm Maturation, and Means of Inducing Reversible Infertility in the Male.
Contractor: Harvard Medical School
Money Allocated: $66,656 (FY 69 Funds), $59,944 (FY 70 Funds), $68,151 (FY 71 Funds)

Objectives: This research is centered upon the use of modern, high quality ultrastructural techniques to investigate key issues of testicular and spermatozoal morphology function. One project deals with topics in spermatogenesis. The investigators are studying the significance of the chromatoid body and aggregates of spermatocytes within the seminiferous tubules, electron microscope radiographic studies of RNA and protein synthesis in spermatocytes and spermatids and, by the use of mitotic blocking agents, interfering with specific developmental events in spermiogenesis in order to learn more about the function of micro-organelles found in germinal cells.

Major Findings: By use of a small tracer, lanthanum nitrate, and high resolution microscopy, two components of the blood-testis barrier have been observed: (1) an adventitial component residing in the cell junctions of the myoid cell layer and (2) an intraepithelial component residing in junctional specializations on the interface between adjacent Sertoli cells. The blood-testis barrier is lacking at birth; it appears shortly after initiation of spermiogenesis when the tubles first acquire a lumen.

Preliminary experiments indicated that λ-chlorhydrin treated rats have many fewer active sperm with qualitative differences in the character of the tail movements.

After treatment with WIN 18446 there is an increased degeneration of spermatocytes and a coalescence of cap phase spermatids in such a way that their acrosomes fuse but the nuclei remain separate. The correlation of onset of abnormalities of nuclear shape with onset of nuclear condensation suggests the possibility that the drug may selectively interfere with this process supporting the conclusion from comparative studies of normal spermiogenesis that the shape of the sperm nucleus is not determined by external forces but by the pattern of aggregation of complexes of DNA with species-specific, arginine-rich histones synthesized during nuclear condensation.

Significance to Biomedical Research and Program of the Institute: Studies of these topics are deemed highly relevant to the purposes of the contraceptive development program.

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July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2107
Contract Title: Morphological and Experimental Studies on Spermatogenesis, Sperm Maturation, and Means of Inducing Reversible Infertility in the Male.
Contractor: Harvard Medical School

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2137
Contract Title: Role of the Epididymis in Sperm Maturation
Contractor: Pennsylvania State University
Money Allocated: $30,706 (FY 69 Funds), $58,660 (FY 70 Funds), $65,359 (FY 71 Funds)

Objectives: The specific objectives are: (1) to determine actual sperm production rate in bulls; (2) to compare ultrastructure and biochemical characteristics of spermatozoa recovered by cannulation of the rete testis with those of cells recovered by cannulation of the vas deferens; (3) to characterize biochemically the testicular effluent and, by comparing this with seminal plasma and accessory gland fluids, to establish the nature of the epididymal secretion; and (4) to determine if there is a substance in the testicular effluent which might exert a local control on spermatogenesis or epididymal function.

Major Findings: The comparison of the fluids from the successful rete testis cannulae and the vas deferens cannulae seems to indicate that 98% of the fluid produced by the testis is resorbed by the epididymis and that the number of sperm leaving the testis and the vas deferens are essentially the same, at least under those experimental conditions.

Biochemical studies have been performed on the fluids collected. Sixteen free amino acids were identified in rete testis fluid, vas deferens plasma, accessory gland fluid, and seminal plasma. Glutamate was the predominant amino acid in all fluids with the exception of the accessory gland fluid. There is a marked decrease in relative concentration of alanine and glycine between the rete testis and the vas deferens fluids. This may suggest selective reabsorption of amino acids by the epididymis. Free myo-inositol is present in the rete testis fluid and is apparently in higher concentration in rete testis sperm. Glutamate is metabolized by sperm from the rete testis, vas degerens, and the ejaculate. The oxidation of C^{14} glutamate or arginine to CO^2 is severly depressed by rete testis fluid.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies directed towards the development of new male contraceptives.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2139
Contract Title: Ultrastructural and Electron Cytochemical Studies of Mammalian Spermiogenesis
Contractor: The University of Texas
Money Allocated: $35,875 (FY 69 Funds), $58,230 (FY 70 Funds), $64,282 (FY 71 Funds)

Objectives: The research project involves studies of: (1) the formation of the manchette in the spermatids during spermiogenesis, by electron microscopy and cytochemistry at the electron microscopic level (ATPase, succinic dehydrogenase, alkaline and acid phosphatases, thiamine phosphatase, aryl sulfatase and β-glucuronidase); (2) the inhibiton of microtubule formation in the manchette (colchicine, cold shock, inhibitors of protein and RNA synthesis); (3) the ultrastructure and cytochemistry of seminal vesicles, bulbourethral gland and prostate.

Major Findings: The study of the effect of Colcemid was continued and extended to mice and to hamsters which were allowed to recover for various periods of time following Colcemid treatment. The findings of the first year of the contract were confirmed: in all hamsters injected with a sublethal dose of Colcemid, damage to the spermatids was apparent after 19 days of treatment. Cessation of the treatment resulted in partial regeneration of the tubules (32-90%) in 2 hamsters. Preliminary experiments in mice indicate the same type of effect of Colcemid on spermiogenesis but at a much higher dose (still sublethal). The toxicity of β-mercaptoethanol in the hamster has been established and the effect of sublethal doses on the meiotic and mitotic index in the bone marrow and testis was studied. The data suggest that β-mercaptoethanol inhibition of cell division is confined primarily to the testis.

The investigators have found that thiamine pyrophophatase and acid phosphatase diminish in the Golgi cisternae when the organelle migrates in the developing spermatid. A number of other enzymes have been localized at the E-M level in the testis. The normal study of the morphology, histochemistry and ultracytochemistry of the ductuli efferentes has been completed and that of the epididymis initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch regarding the development of new male methods of contraception

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2162
Contract Title: Biochemical Studies on Spermatogenesis and Spermatozoa
Contractor: Northwestern University
Money Allocated: $39,944 (FY 69 Funds), $45,061 (FY 70 Funds), $45,061 (FY 71 Funds)

Objectives: The applicant has published extensively on lactate dehydrogenase isozymes. Relevant to the current proposal are his studies on the LDH-X isozyme found in testis and spermatozoa. The applicant is now studying the role of this isozyme in spermatogenesis and the metabolic economy of epididymal and ejaculated spermatozoa, and determining whether other testis-specific forms of other enzymes exist and what part they may play in the biochemistry and function of the male reproductive system. The applicant asks the question, does LDH-X have any functional role in sperm production, motility or functional capacity? His immediate approach to such studies is to isolate sufficient quantities of the LDH-X isozyme to produce antibodies to this enzyme. Mouse testes are used as the source for the enzyme and antibodies are raised in rabbits. These antibodies to the LDH-X and other subunits, e.g., LDH-1 and LDH-5, will be used to isolate LDH labeled during biosynthesis in the animal using radioactive amino acids. From these data, isozyme synthesis rates or isozyme half-life can be calculated.

Major Findings: The investigator has isolated a quantity of LDH-X and has raised antibodies to the preparation. A relatively simple technique has been devised to purify the isozyme which essentially involves ammonium sulfate fractionation and sephadex filtration. Data is presented to show that injection of the antibody to the isozyme induced infertility in male mice. The curve of infertility shows a drop of about 60% during the third week of treatment which begins to return to original fertility level by the fourth week of treatment. Injection of antibody produced about one week of partial infertility.

Significance to Biomedical Research and Program of the Institute: It is important to establish LDH isozymes turnover rates in the testes since this information is basic to the complete understanding of the normal function of the testes. Studies of this sort are highly relevant to the purposes of the contraceptive development program.

Proposed Course: This continuing contractual effort leading toward the development of new contraceptives is an integral part of a targeted research program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2166
Contract Title: Hormonal Control of Sperm Maturation
Contractor: Vanderbilt University
Money Allocated: $27,527 (FY 69 Funds), $37,083 (FY 70 Funds), $50,394 (FY 71 Funds)

Objectives: This research is directed towards establishing the hormonal factors involved in sperm maturation in the epididymis. The investigator has undertaken a series of endocrine interventions, including ablation and replacement experiments in order to study the effects of these procedures on the fertilizing capacity of epididymal sperm of the male rabbit.

Major Findings: Spermatozoa leaving the testis are immature and unable to fertilize ova. Fertilizing ability is attained when spermatozoa arrive in a definite epididymal segment: the lower part of the corpus. To pass from a state of complete unfertilizing capacity (in the upper corpus) to a state where they are able to fertilize ova, spermatozoa require approximately twelve hours. To investigate the hormonal dependance of the epididymal function, the investigator studied effects of castration and hypophysectomy on the development of sperm fertilizing ability. Hypophysectomy resulted in a significant loss of fertilizing capacity from the lower part of the corpus of the rabbit epididymis. Although spermatozoa did not become fertile their motility appeared to be unimpaired.

Rabbits were hypophysectomized and given testosterone replacement for seven days. The androgen appeared to prevent the loss of fertility resulting from hypophysectomy.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new, male methods of contraception.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 69-2220
Contract Title: Morpho-Physiologic Studies on Mammalian Gametes Prior to and During Fertilization In Vivo and In Vitro
Contractor: Harbor General Hospital
Money Allocated: $23,783 (FY 69 Funds), $42,416 (FY 70 Funds), $45,978 (FY 71 Funds)

Objectives: This work involves light and electron microscope studies of gametes, especially the acrosomal changes in fertilizing sperm and the cortical granules of eggs prior to and during fertilization. These techniques will also be used to determine the number of sperm around mouse eggs at the time of fertilization, and whether it is the first sperm, or another among the many in the vicinity, that fertilizes the egg. Sperm transport in the uterus and the oviduct and egg transport in the oviduct are also being investigated.

Major Findings: The investigator has demonstrated that passage of mammalian sperm into the oviduct occurs very early after coitus and that the utero-tubal junction is important in the transfer of spermatozoa from the uterus to the oviduct, allowing for only a small fraction of the total ejaculated spermatozoa to enter the oviduct. Control is effected not by a persistently narrow lumen of the utero-tubal junction, but by intermittent closing of this structure. The mechanism involved in this closure remains to be elucidated. Evidence has been obtained that of the total number of sperm shed by the mouse at coitus, only those which enter the tubal lumen very soon after copulation may participate in fertilization. Since the transit to the fallopian tube occurs within minutes after coitus, the investigator proposes that if capacitation does occur in the mouse, it takes place in the tubal lumen, rather than the uterus, and involves only a limited fraction of the total number of shed spermatozoa.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch as it pertains to the mechanisms involved in the critical process of fertilization.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2056
Contract Title: Composition of Epididymal Plasma and Sperm in the Boar and in Man
Contractor: Royal Veterinary College
Money Allocated: $10,316 (FY 70 Funds)

Objectives: If, as it would appear from published data, the compound chloropropanediol inhibits fertility in male rats within 10 days of administration, it must be acting on spermatozoa after they have entered the epididymis. It then follows that this antifertility agent acts via the epididymal secretion. Thus, it should be possible to show that chloropropanediol affects the composition of epididymal plasma as taken from the various segments. For this purpose the investigator will use initially the boar epididymis, and later the human one to test whether the drug causes infertility. If it does, epididymal plasma and sperm will be analyzed before and after administration of the drug. Studies on ionic composition and lipids in normal boars and in the testis and epididymis from men undergoing castration for various reasons will also be conducted.

Major Findings: The drug 3-chloro-1,2,propanediol administered to boars produced changes in sperm motility and rendered the spermatozoa infertile.

The inuline space in epididymal and ejaculated spermatozoa is different.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of sperm transport, capacitation, and survival in the reproductive tract of male and female animals.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30 1970

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2143
Contract Title: The Maturation of Mammalian Sperm in the Male and Female Reproductive Tract
Contractor: Columbia University College of Physicians and Surgeons
Money Allocated: $72,770 (FY 70 Funds), $22,998 (FY 71 Funds)

Objectives: The first objective of this research project is to study the process of sperm maturation in the male genital tract: (1) by ultrastructural studies of the morphological changes occurring in the acrosome during spermiogenesis in the human; (2) by an investigation of changes in the sperm plasma membrane during epididymal maturation in normal and ligated rabbit epididymides, and in the normal monkey and human epididymis; (3) by an investigation of the development of sperm motility in the primate and human epididymis; (4) by studies in rats and rabbits of the genetic consequences of spermatogenic inhibition or of disruption of epididymal function during the period of declining fertility in order to assess the risk of abnormal offspring after treatment with antifertility agents.

A second objective is a study in the female of the factors affecting the sperm acrosome reaction: (1) by characterizing chemically the decapacitation factor in bull and rabbit seminal plasma; (2) by studying the influence of the rabbit seminal plasma decapacitation factor on the acrosome reaction of sea urchin sperm; (3) by studying the influence of follicular fluids on the sperm head plasma membrane/acrosome complex of rabbit primate and human spermatozoa; and (4) by studying the structural responses of human and primate sperm to homologous oocytes matured in vitro.

Major Findings:
1. Unlike the situation in other mammals, no significant changes occur in the form of the human acrosome from the early phase of its development through later stages of its association with the Sertoli cell and release and passage into the epididymis. The only exception is the formation of the equatorial segment in the very late spermatid.
2. Studies on the sperm plasma membrane indicate that during the passage of sperm through the epididymis surface charges are constantly altered. These results demonstrate clearly the existence of radical changes in the chemical architecture of the sperm surface during maturation in the epididymis.
3. Studies on human sperm motility indicate that a majority of human sperm appear not to acquire the capacity for normal progression until they reach the lower corpus.
4. In male rabbits treated with large doses of a long acting estrogen one observes effects on epididymal sperm before gross effects on sperm production can be seen. Initial observation in changes in sperm morphology was not correlated with infertility which took place somewhat later.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2143
Contract Title: The Maturation of Mammalian Sperm in the Male and Female Reproductive Tract
Contractor: Columbia University College of Physicians and Surgeons

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies devoted to elucidating the factors in the male and female reproductive tract responsible for the various phases of sperm maturation and the development of fertilizing capability.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2147
Contract Title: Enzymes of the Sperm Acrosomes, Occurrence of the Inhibitors of These Enzymes in Seminal Plasma and the Role of the Enzymes and Inhibitors in Capacitation of Sperm and in the Penetration and Fertilization of Ova
Contractor: University of Georgia
Money Allocated: $29,494 (FY 70 Funds), $45,835 (FY 71 Funds)

Objectives: The objectives of this research project are: (1) to isolate and purify the five acrosomal enzymes from mammalian sperm; (2) to demonstrate their role in fertilization; (3) to elucidate the role played by enzyme inhibitors in seminal plasma (including decapacitation factor, DF); (4) to attempt to correlate DF activity with the inhibition of a new sperm neuraminidase; and (5) to study uterine enzymes and their possible role in capacitation.

Major Findings: A trypsin-like enzyme from the sperm acrosome has been named "acrosin" since it resembles plasmin as well as trypsin. This enzyme has been purified 250-fold and is 90% pure by disc electrophoresis. Acrosin has a molecular weight of 55,000 as estimated by Sephadex chromatography and consists of two equal size subunits. The biochemical properites of the enzyme have also been studied. Acrosin has been demonstrated to be inhibited by several polypeptide inhibitors including seminal plasma trypsin inhibitor as well as synthetic trypsin inhibitors. Treatment of live sperm with such inhibitors prevents fertilization.

A sperm neuraminidase (SN) has been obtained from the acrosomal neuraminidase-like factor (NLF). The biological role of sperm neuraminidase has been demonstrated. The zona pellucida of rabbit ovum is rendered resistant to dissolution by mercaptoethanol and digestion by trypsin after pre-treatment with the sperm neuraminidase. Similarly, pre-treatment with SN renders rabbit ova completely unfertilizable.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the role of sperm acrosomal enzymes and their inhibitors in capacitation and fertilization.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2152
Contract Title: An Evaluation of the Efficacy of Testosterone-Filled Dimethylpolysiloxane Implants to Maintain Varying Concentrations of Testosterone in the Peripheral Circulation of Male Rabbits
Contractor: Oklahoma State University
Money Allocated: $28,917 (FY 70 Funds), $14,459 (FY 71 Funds)

Objectives: The objectives of this research project are to study: (1) The diffusion of labeled testosterone from dimethylpolysiloxane (DPS) capsusules in vitro in distilled water in order to establish the appropriate capsule dimensions for a spectrum of 24 hour diffusion rates in vivo; (2) the effects of various capsular dimensions on the concentration of tritiated testosterone in the peripheral plasma of male rabbits; (3) the effects of implanting tritiated testosterone-filled DPS capsules into various anatomical sites on the concentration of tritium in the peripheral plasma of male rabbits; and (4) study the efficacy of DPS testosterone implants for releasing and maintaining peripheral testosterone concentrations at some predetermined level for 6 months in intact and castrated male rabbits.

Major Findings: Initial experiments of Drs. Ewing and Desjardins have shown that tritiated testosterone passes unaltered from DPS capsules into water. The release rate from capsule is dependent upon the capsule surface area. The release from capsules implanted either subcutaneously or intraperitoneally is similar and is dependent upon capsule area. And finally only 1-2% of the tritiated material present in peripheral plasma of male rabbits containing subcutaneous testosterone tritium-filled DPS implants has the same chromatographic mobility as authentic testosterone when subjected to thin layer chromatography.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to develop new methods of systemic or local administration of contraceptive agents in both the male and female.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

Terminal 6 months.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2156
Contract Title: Control Mechanisms of Sperm Motility
Contractor: State University of New York
Money Allocated: $30,836 (FY 70 Funds), $38,170 (FY 71 Funds)

Objectives: The applicant proposes that sperm motility is the result of a control system consisting of a free-running oscillator, an amplifier, a power supply, and a feedback loop. The investigator proposes a number of novel experiments designed to investigate the nature of this control system and its components. Specifically, he proposes: 1) To measure the electrical activity of the oscillator in the centriole by inserting glass microelectrodes into the head of sperm immobilized first by low temperature, and to search for electrical potentials of the same frequency as the tail wave. 2) To destroy by a laser beam, areas of the centriole. A glass microelectrode will then be inserted into the head of the immobilized sperm to monitor the electrical activity, and to attempt electrically to reactivate the mechanism for motility. 3) To inhibit the action of the oscillator and its coupling, by specific chemicals such as xylocaine and procaine. 4) To investigate the feedback mechanism by inhibiting respiration, and then measuring the change of frequency of the tail wave.

Major Findings: Measurement of potential of the sperm head by an impaled microelectrode gave a negative potential of -7mV at pH 7. This potential is a Donnan potential caused by fixed charges, (not a membrane potential) is reproducible and can be measured from detached sperm heads. Potential due to a distribution of mobile ions can not be measured with presently available techniques. The possibility of measuring "action potentials" in sperm seems at present to be excluded.

Impaled sperm can be stimulated electrically or through the addition of ATP to the surrounding medium. Otherwise the impalement renders them immobile.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies in which electrophysiological techniques are applied to elucidating complex reproductive processes such as sperm motility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2162
Contract Title: Sperm Capacitation as Target for Contraception: Animal Studies
Contractor: Vanderbilt University
Money Allocated: $44,278 (FY 70 Funds), $44,278 (FY 71 Funds)

Objectives: The objective of this research project is to perform animal studies aimed at clarifying the role of leukocytes in the capacitation process and improving the methodology for the study of capacitation. Alkylating agents or extracorporeal irradiation of blood will be used to produce systemic leukopenia in an attempt to eliminate leukocytes from the endometrium and uterine lumen. Preliminary experiments using castrated, estrogen-primed rabbits treated with nitrogen mustard demonstrated a reduction in the number of leukocytes contaminating suspensions of sperm removed after uterine incubation. The investigator hopes to reduce leukocytes further by administration of suppressing agents before estrogen priming. Epididymal sperm will be incubated in the uteri of ovariectomized, leukocyte-depleted, estrogen-primed rabbits, and the effects of incubation in this environment will be tested by in vivo and in vitro fertilization assays. In addition, C^{14}- or H^3-labeled nitrogen mustard will be used in radioautographic studies to determine whether alkylation of endometrial components may be a factor in the inhibition of capacitation. Finally, ultrastructural studies will be conducted on the invasion of fertilized ova by leukocytes.

Major Findings: Colchisine treatment of the capacitators inhibits the leukocytic response of the uterus to sperm. However, sperm recovered from leukopenic uteri maintain their fertilizing capacity when transferred to recipient females. Likewise, insemination of colchisine treated females results in normal fertilization rates.

Studies with nitrogen mustard induced leukopenia suggest that addition of polymorphonuclear leukocytes to sperm suspensions facilitates in vitro capacitation in leukopenic rabbits. These studies are of a very preliminary nature and need repetition. Initial experiments have been conducted on the distribution of the alkylating material. It appears to be distributed randomly in the body in all compartments of the endometrial cell.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on new methods of studying complex reproductive functions such as sperm capacitation.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2167
Contract Title: Sperm Transport and Survival in the Human Female Genital Tract
Contractor: University of Michigan Medical Center
Money Allocated: $32,723 (FY 70 Funds), Terminated

Objectives: The objective of this research project is to study the rate of transport and survival of sperm deposited at the cervix of women prior to surgical removal of the genital tract. Sperm, recovered from various parts of the tract by flushing, will be counted and studied for motility, morphological changes, and fluorescence after exposure to tetracycline-HCl. Tissues from various segments of the reproductive tract will be mixed with semen to observe their effects on sperm motility and agglutination. The investigator hopes to study the rate of sperm transport and length of survival of sperm at various stages in the menstrual cycle. By means of morphologic changes he proposed to clarify the role of capacitation in humans. Semen will be applied to the cervix at various intervals before hysterectomy. At least six groups of hormonally different women will be studied.

Major Findings: In 21-22 hrs after artificial insemination, only small numbers of spermatozoa are found in the cervix or the oviducts.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of sperm transport, capacitation, and survival in the reproductive tract of human females.

Proposed Course: Renewal not requested

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2253
Contract Title: Ultrastructural and Immunological Investigation of Spermatogenesis, Sperm Transport, and Fertilization in the Mammal
Contractor: University of Miami
Money Allocated: $92,300 (FY 70 Funds), $58,275 (FY 71 Funds)

Objectives: The objective of this research project is to study the effects of antibodies on spermatozoa in the female tract of mammals, using bivalent and univalent fragments of guinea pig and goat anti-rabbit semen antibody.

The investigators will examine the fertilizing capacity and transport of antibody-pretreated spermatozoa following intrauterine artificial insemination, in an attempt to establish whether antibodies can inhibit spermatozoan passage from the uterus through the tubes to the site of fertilization. In addition, intratubal and/or in vitro insemination experiments with univalent and bivalent antibody-treated spermatozoa will be performed in an attempt to determine if antibody treatment inhibits capacitation and whether antibody can inhibit the fertilizing capacity of capacitated sperm. Anti-sperm antibody inhibition of sperm head enzymes thought to be responsible for ovum penetration will be tested by preparing extracts of these enzymes and examining their activity on appropriate substrates in the presence of univalent and bivalent antibodies.

Major Findings: Spermatozoa treated with anti-semen antibody were inseminated intravaginally. Subsequent examination of rabbit ova showed that none were fertilized. Intrauterine insemination of antibody treated rabbit spermatozoa resulted in a marked decrease in fertilizing capacity (11% vs 68% in controls). It is still unknown at what level did fertility failure occur. Intratubal inseminations are now being conducted.

Papain digested (univalent) rabbit antisemen-antibody inhibited the capacity of rabbit semen to disperse the cumulus oophorus mass surrounding newly ovulated rabbit eggs. This action may be the result of inhibition by the antibody of sperm hyaluronidase. Hyaluronidase from commercial sources is not inhibited by anti-rabbit semen antibody.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies which utilize immunological techniques to study reproductive processes.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2262
Contract Title: Mechanism of Action of Gonadotrophins in the Testes
Contractor: University of Georgia
Money Allocated: $33,500 (FY 70 Funds)

Objectives: The objective of these studies is to confirm and extend the applicant's observations of the mechanism of action of FSH and ICSH in testicular tissue. These original observations include a stimulation of protein and fatty acid synthesis in vitro by nuclear RNA (N-RNA) or polysomes isolated from testes removed from FSH-ICSH-treated, hypophysectomized, immature rats. The investigators will determine by time course and inhibitor studies if protein synthesis is a prerequisite for fatty acid synthesis in the in vitro system, will further purify the cytoplasmic fraction and determine the biochemical changes induced in it by gonadotrophins, and will isolate and identify the fatty acids and other lipids synthesized as a result of gonadotrophin treatment.

Major Findings: As a result of administering the gonadotropins FSH-LH to mature male hypophysectomized rats the following observations have been made in vivo.

1. An increase in both wet and dry weight results.
2. A rapid increase in protein in mg per testis occurs.
3. An increase in C^{14}-orotic acid incorporation into total cellular RNA expressed as total counts per minute (CPM) and CPM per testis is observed.
4. Separation of the RNA into various fractions reveals that both cytoplasmic and nuclear RNA (N-RNA) have increased in total CPM and CPM per testis.
5. C^{14}- orotic acid incorporation into polysomes increases and further studies show that the ribosomal RNA (rRNA) is the major species labeled in the polysomes.
6. An increase in DNA content in mg per testis occurs and indication that cell division has been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the influence of hormonal environment on testicular synthetic processes.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2313
Contract Title: Neurochemical Control of Sperm Motility
Contractor: Medical College of Ohio
Money Allocated: $45,558 (FY 70 Funds), $45,558 (FY 71 Funds)

Objectives: The investigator will apply biochemical, electron microscopic, and electrophysiological procedures to the study of control of flagellar movement as related to sperm motion. More specifically the applicant will conduct a systematic analysis of the role of acetycholinesterase in the regulation of the propulsive motion of spermatozoa. The study will be conducted in three phases:

1. Sperm cell suspensions will be subjected to microcolorimetric analysis of acetycholinesterase activity, rating of motility, timing of swimming duration, electron microscopic cytochemical procedures, and microelectrode determinations of electrical potentials. The effects of specific acetycholinesterase inhibitors, such as eserine, on sperm propulsion will also be studied.

2. In the second part, the investigator will examine the developmental and maturational aspects of flagellar growth in relation to the development of sperm motility.

3. The third part will be directed toward the environmental interactions, both physical and chemical, between the sperm cells and the medium.

Major Findings: Dr. Nelson's studies are directed toward the elucidation of the role that the acetylcholine-acetycholinesterase system plays in sperm motility. The following information has been obtained to date. Analysis of the enzyme's localization place enzymatic activity in mitochondria located in the flagellar midpiece. Enzymatic activity has not been observed in other sections of the spermatozoa. Inhibition of enzyme activity completely prevents the deposition of reagents that are used for enzyme localization (visualization).

One of acetycholine's presumed modes of action involves membrane depolarization and consequently alterations in permeability. Measurment of electrical parameters gives an insight of the magnitude of these alterations. The investigators were successful by using glass microelectrodes in measuring a negative (internal) potential from implaed bull spermatozoa. The magnitude of this potential was directly related to sperm activity. Being higher in actively flagellating sperm and lower in spermatozoa exhibiting little activity through the use of "high K^{+}" media a state of membrane hyperpolarization has been achieved in bull spermatozoa. Forward motility is abolished and only twitching is retained.

Contract Number: 70-2313 (page 2)

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on sperm motility.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT

July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2315
Contract Title: Macromolecular Biosynthesis in Epididymal, Ejaculated, and Capacitated Spermatozoa
Contractor: Worcester Foundation for Experimental Biology, Inc.
Money Allocated: $52,785 (FY 70 Funds)

Objectives: The investigator will study macromolecular biosynthesis in sperm by extraction and partial characterization of the protein synthesized and determinate if the radioactive uptake is due to repair of histones or de novo synthesis. Initially, the investigator will study amino acid incorporation and then test the effect of radioactive carbohydrates on synthesis of TCA-in soluble material. Future plans relate to the use of accelerators and inhibitors, the study of enzyme reactions and the chemistry of the synthesized protein. Epididymal and capacitated sperm will be studied in addition to ejaculated sperm. The protein-synthesizing system will be used as a tool for testing antifertility compounds.

Major Findings: Dr. Hele has been investigating the incorporation of radioactive amino acids into the trichloroacetic acid insoluble fraction by ejaculated rabbit and bull spermatozoa. Histidine, phenylalanine, lysine, and arginine were incorporated at a high rate, whereas minimal incorporation of threonine, valine and glutamic acid was noted. Bull sperm incorporated the amino acids at a higher rate than did rabbit sperm. Bacterial contamination of the incubates can not account for the protein synthesis observed. Attempts were made to estimate the amount of RNA in spermatozoa. Small amounts of RNA may be present but the data are equivocal at best. Extraction procedures for the determination of the nature of the labelled material have been devised. In a preliminary experiment, actinomycin, cycloheximide, and puromycin failed to inhibit the incorporation of ^{14}C-phenylalanine plus ^{14}C-histidine in ejaculated rabbit spermatozoa.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on macromolecular biosynthesis of spermatozoa.

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2316
Contract Title: The Influence of Oviduct Secretions on Oxidative Phosphorylation of Spermatozoa
Contractor: University of Georgia
Money Allocated: $12,300 (FY 70 Funds), $6,162 (FY 71 Funds)

Objectives: The investigator plans to study the effect of tubal secretions on oxidative phosphorylation of boar spermatozoa using 2-deoxyglucose as the phosphate acceptor to determine P:O ratios. Since spermatozoa in the female reproductive tract tissues depend largely on aerobic, rather than anaerobic metabolism, this study is designed to give a deeper insight into the metabolic patterns of sperm in the female tract.

Major Findings: Calculations of P/O ratios were undertaken in 10 experiments. The observed P/O ratios for the intact sperm ranged from 0.74 to 2.12 with a mean of 1.46. These values are lower than one observes in cell homogenates.

Addition of oviductal fluid from estrous sows to the incubation medium increase both oxygen utilization and P uptake with resulting P/O ratios of 2.26 and 2.62 (two experiments). These two values appear to be higher than the control mean.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the metabolism of spermatozoa.

Proposed Course: Termination after 6 months.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number: 70-2317
Contract Title: Protease Inhibitors in Human Genital Secretions
Contractor: The University of Chicago
Money Allocated: $23,227 (FY 70 Funds), $11,614 (FY 71 Funds)

Objectives: The investigator will study the possibility of a protease-inhibitor relationship of biological significance in the interaction between seminal fluid and sperm on the one hand and female genital tract secretions on the other. Alpha-1-antitrypsin has been discovered in cervical mucus and its level is governed by sex hormones. Other inhibitors may also be present in seminal fluids and in the female tract.

Major Findings: Proteolytic activity of acrosomal extracts has been tested in gel diffusion systems containing denatured hemoglobin, heat denatured bovine fibrinogen, elastin and gelatin. Only gelatin shows some degree of susceptibility to the acrosomal enzyme.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on sperm inhibitors.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

Terminal 6 months.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Testis and Sperm Specific Antigens of Man and Certain Other Primates
Contractor: University of Michigan
Money Allocated: $21,140 (FY 71 Funds)

Objectives: Immunization with testis and sperm antigens is a possible means of human contraception. However, the use of the materials will depend upon knowledge of the characterization and purification of these antigens. This proposal is intended to yield information on the chemical and immunologic nature of immunogens specific for testis and sperm, the possible influence of the epididymis on the antigenic molecules, and the effects of antibodies against these antigens on sperm viability and function. The use of immunesera from infertility patients will permit detection of those testes and sperm antigens that are immunogenic in man and their possible relationship to infertility. These immunogens would be purified and characterized. The effects of antibodies induced by the different antigens upon sperm will be determined by laboratory tests involving agglutination, immobilization and cervical mucus penetration. Possible changes in the chemistry of sperm antigens in the epididymis will be associated with the maturation of sperm cells. After characterization and purification of the immunogens future plans call for isoimmunization of male and female monkeys to determine the effects on the fertility process.

Significance to Biomedical Research and Program of the Institute: Immunologically induced infertility could be a useful contraceptive method and its study is highly relevant to the contraceptive development program especially since the work will be done on primate material.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Contraceptive Development Studies for Males: Oral Steroid Hormone Administration
Contractor: University of Washington School of Medicine
Money Allocated: $58,863 (FY 71 Funds)

Objectives: This contract proposal is designed to evaluate the combined effects of two androgenic steroids on spermatogenesis when administered to normal adult human males. The ultimate goal is to develop an effective oral contraceptive technique for males.

At the present time there is no compound available for effectively inhibiting human spermatogenesis which is non-toxic or absent from adverse reactions which would render the preparation either unacceptable to the patient or to his physician.

Significance to Biomedical Research and Program of the Institute: Development of contraceptive techniques for the male is highly relevant to the contraceptive development program.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Synthesis and Development of a Potent Progestational Steriod for Use as a Contraceptive
Contractor: Syntex Research, Division of Syntex
Development Research Laboratory
Palo Alto, California
Money Allocated: $35,000 (FY 71 Funds)

Objectives: The purpose of this project is to synthesize a novel steroidal compound and test its progestational and antifertility effect in rabbits and dogs. Because of the confidential nature of this aspect of the CDB program, disclosure of the chemical structure of the specific compounds cannot be made until release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to have this novel steroidal compound synthesized and tested for progestational and antifertility activity as this work is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Synthesis, Characterization, and Biological Testing of Specifically Designed Anti-implantational Agents
Contractor: Boston University
Department of Biochemistry
Boston, Mass.
Money Allocated: $57,500 (FY 71 Funds)

Objectives: The purpose of this project is to synthesize a series of steriodal and non-steroidal compounds and to test them for antifertility activity in rats, rabbits, and hamsters. Because of the confidential nature of this aspect of the CDB program, disclosure of the chemical structure of the specific compounds cannot be made until release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to test these steroidal and non-steroidal compounds as antifertility agents as this work is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Synthesizing and Testing of Antifertility Compounds.
Contractor: Collaborative Research, Inc.
Waltham, Mass.
Money Allocated: $138,704 (FY 71 Funds);for two years

Objectives: It is the purpose of this project to synthesize a series of polyheterocyclic compounds and to test their antifertility effect in mice and rats. Because of the confidential nature of this aspect of the CDB program, disclosure of the chemical structure of the specific compounds cannot be made until release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to have these polyheterocyclic compounds synthesized and tested for antifertility activity as this work is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: The Provision and Synthesis of Certain Alkylating Steroids and the Testing of Their Possible Antifertility Effects
Contractor: Southern Illinois University
Department of Physiology
Carbondale, Illinois
Money Allocated: $24,899 (FY 71 Funds)

Objectives: It is the purpose of this project to synthesize and test several steroidal compounds for antifertility activity in mice. Because of the confidential nature of this aspect of the CDB program, disclosure of the specific compounds cannot be made unless release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to have these steroidal compounds synthesized and tested for antifertility activity as this work is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Synthetic Antifertility Compounds
Contractor: Stevens Institute of Technology
Department of Chemistry and Chemical Engineering
Hoboken, New Jersey
Money Allocated: $54,648 (FY 71 Funds)

Objectives: It is the purpose of this project to synthesize a series of polycyclic, polyheterocyclic, and steroidal compounds and to test their antifertility effect in rodents. Because of the confidential nature of this aspect of the CDB program, disclosure of the chemical structure of the specific compounds cannot be made until release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to have these polycyclic, polyheterocyclic and steroidal compounds synthesized and tested for antifertility activity as this work is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: The Synthesizing, Furnishing and Testing of Antifertility Compounds
Contractor: Research Triangle Institute
Chemistry and Life Sciences Laboratory
Research Triangle Park, North Carolina
Money Allocated: $114,600 (FY 71 Funds)

Objectives: The purpose of this project is to synthesize a series of steroidal and non-steroidal compounds and to test their antifertility effect in mice. Because of the confidential nature of this aspect of the CDB program, disclosure of the chemical structure of the specific compounds cannot be made until release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to have these steroidal and non-steroidal compounds synthesized and tested for antifertility activity as this work is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Synthesis and Antifertility Testing of Prostanoic Acid Derivatives
Contractor: Ayerst Laboratories
Department of Pharmacology
Montreal, Canada
Money Allocated: $265,200 (FY 71 Funds);for two years

Objectives: The purpose of this project is to synthesize a series of prostanoic acid derivatives and to test their antifertility effect in rats. Because of the confidential nature of this aspect of the CDB program, disclosure of the chemical structure of specific compounds cannot be made until release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to have these prostanoic acid derivatives synthesized and tested for antifertility activity as this work is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Investigation of the Antifertility Activity in the Monkey and Long-Term Biological Effects in the Rat, Dog and Monkey of a Novel, Synthetic Steroid.

Contractor: Lederle Laboratories Division, American Cyanamid Company
Metabolic Disease Therapy Research Section
Pearl River, New York
Money Allocated: $75,000 (FY 71 Funds)

Objectives: The purpose of this project is to synthesize a novel steroidal compound and to test its antifertility effect in rats, rabbits and monkeys. Because of the confidential nature of this aspect of the CDB program, disclosure of the chemical structure of the specific compound cannot be made until release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to have this steroidal compound synthesized and tested for antifertility activity as this work is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Synthesis and Testing of Antifertility Compounds
Contractor: Stanford Research Institute
Department of Pharmaceutical Chemistry
Menlo Park, California
Money Allocated: $108,395 (FY 71 Funds)

Objectives: The purpose of this project is to synthesize a series of steroidal and heterocyclic compounds and test their antifertility effect in rats. Because of the confidential nature of this aspect of the CDB program, disclosure of the chemical structure of the specific compounds cannot be made until release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to have these steroidal and heterocyclic compounds synthesized and tested for antifertility activity as this work is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: The Use of the Bionyx Control as a Contraceptive (Reversible Vasectomy Device) in Man
Contractor: New York Medical College
Department of Pharmacology
New York, New York
Money Allocated: $50,000 (FY 71 Funds)

Objectives: The purpose of this project is to ascertain the usefulness of a reversible intravasal valve as a contraceptive device in the male guinea pig. Because of the confidential nature of this aspect of the CDB program, disclosure of the nature of the device cannot be made until release of information will be allowed.

Significance to Biomedical Research and Program of the Institute: It would be important to determine the usefulness of this contraceptive device as the development of devices for male sterilization is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Research and Development of Contraceptive Devices and Materials. Development of New Sterilization Techniques
Contractor: Abcor, Inc., Cambridge, Massachusetts
Money Allocated: $345,000 (FY 71 Funds); for two years

Objectives: The purpose of this project is to develop (1) a reversible intravasal plug with a hollow center into which a removable pin can be inserted, and (2) an absorbable intravasal stent that would facilitate reanastomosis of the cut end of the vas following vasectomy. A variety of materials will be evaluated for the devices and the effectiveness of these new sterilization techniques will be studied in the guinea pig.

Significance to Biomedical Research and Program of the Institute: It would be important to determine the utility of these contraceptive devices as the development of devices for male sterilization is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Design and Development of Implantable Contraceptive Devices for Use in the Male
Contractor: IIT Research Institute
Medical Engineering Center
Chicago, Illinois
Money Allocated: $150,000 (FY 71 Funds)

Objectives: The purpose of this project is to conduct a feasibility study on the design of devices suitable for intravasal implantation for the purpose of reversibly occluding the vas. Biological studies will be conducted in animals to determine the reversibility and tissue compatability of the devices.

Significance to Biomedical Research and Program of the Institute: The design and development of implantable devices for male sterilization is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Feasibility Study of Intrauterine Microcapsules as a Prolonged Drug Release Form
Contractor: Battelle Memorial Institute
Money Allocated: $61,200 (FY 71 Funds)

Objectives: The purpose of this project is to investigate the feasibility of employing a microencapsulated drug dose form as an intrauterine antifertility measure. Feasibility will center largely on maintaining microcapsules within the uterus. The drug delivery system would involved the microencapsulation of progestagens, spermatocidal agents, or cervical mucosal thickeners. After microencapsulation of the designed drug agent, these microcapsules would be inserted in the uterus for a controlled and prolonged release of the drug.

Significance to Biomedical Research and Program of the Institute: It would be important to conduct a feasibility study of intrauterine microcapsules as a prolonged drug release form as the development of intrauterine antifertility measures is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integrall part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Grafted Hydrogels in Contraceptive Application
Contractor: Franklin Institute Research Laboratories
Chemistry Laboratory
Philadelphia, Pa.
Money Allocated: $103,600 (FY 71 Funds)

Objectives: The purpose of this project is to prepare hydrogel-coated intra-uterine devices and hydrogel-coated intra-uterine devices impregnated with copper and to study these devices in rabbits to determine their tolerance and effectiveness as antifertility devices. Conventional IUD's have already shown promise and it is anticipated that there will be greater tolerance and less medical problems with a hydrogel-coated IUD than with the conventional IUD. A series of studies will be performed with completed, scaled devices on the rabbit initially and then, based on these animal results, in the human.

Significance to Biomedical Research and Program of the Institute: The development of hydrogel-coated IUD's for female sterilization is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Development of an Intra-uterine Sterilization System for the Female
Contractor: IIT Research Institute
Medical Engineering Center
Chicago, Illinois
Money Allocated: $247,977 (FY 71 Funds)

Objectives: The purpose of this project is to develop a prototype system for intra-uterally sterilizing the female by occlusion of the utero-tubal junction. The occlusive devices would be introduced through the cervical os, and be guided into the tubal location by a specially designed steerable hysteroscope. This would simplify the procedure for the physician or specially trained paramedical personnel, in a non-operative procedure. The design and development effort will be supported with biological and animal experiments and will lead eventually to clinical use in humans.

Significance to Biomedical Research and Program of the Institute: The development of a system for intra-uteral sterilization of the female is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: A Proposal for the Study of a Long Acting Injectable Progesterone Contraceptive
Contractor: Brookdale Hospital Center, Brooklyn, New York
Money Allocated: $116,512 (FY 71 Funds); for two years

Objectives: The purpose of this project is to prepare and evaluate two progesterone contraceptive drug release systems to determine the possibility of designing a progesterone preparation which would release relatively constant amounts of the agent sufficient for contraceptive purposes for at least three months. The two systems involve steroid-lipid pellets and collagen implants. Steroid lipid pellets will be prepared by processing eutectic mixtures of progesterone with various lipids, primarily sterols, in an apparatus permitting preparation of pellets of any desired diameter. Collagen implants will be made by binding progesterone to collagen solution and inducing varying degrees of crosslinking. In vitro and in vivo studies in hamsters and rabbits will be undertaken to determine the rate of dissolution of these different matrices and the rate at which progesterone will be liberated.

Significance to Biomedical Research and Program of the Institute: The development of progesterone drug release systems for female contraceptive measures is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: Device and Technique for Blocking Fallopian Tubes
Contractor: Franklin Institute Research Laboratories
Colloids and Polymers Laboratory
Philadelphia, Pa.
Money Allocated: $78,866 (FY 71 Funds)

Objectives: The purpose of this project is to develop a new device and application technique for accomplishing the blocking of fallopian tubes with medical-grade silicone rubber for reversible sterilization. The device is to be evaluated in rabbits for installation, compatability, antifertility efficacy, and reversibility.

Significance to Biomedical Research and Program of the Institute: The development of a device for accomplishing the blocking of fallopian tubes for female sterilization is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

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Contraceptive Development Branch
Contract and Collaborative Research

Contract Number:
Contract Title: New Hydrophilic Polymers for Population Regulation
Contractor: Polysciences, Inc.
Polymer Laboratories
Warrington, Pa.
Money Allocated: $74,419 (FY 71 Funds)

Objectives: The purpose of this project is to develop new hydrophilic polymers a) for induction of abortion through cervical dilation and b) for extending the life of vaginal spermicidal agents. For induction of abortion using hydrophilic polymers a study of various biologically inert materials will be made in vitro and an experimental study will be conducted in animals. This will be followed by a very limited clinical trial. The study of a substantive hydrophilic vaginal spermatocidal contraceptive will be limited to animals and will ascertain the duration of action of spermatocidal activity as compared to available creams and foams.

Significance to Biomedical Research and Program of the Institute: The development of hydrophilic polymers for induction of abortion and for extending the life of spermicidal agents as a female contraceptive device is directly relevant to the purposes of the CDB activities.

Proposed Course:

This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Evaluation of medical effects of fertility regulating methods represents one of the earliest activities of the Center for Population Research. Although largely devoted to studying the effects of the oral contraceptives, investigations of intrauterine devices have also been under support. During September, 1970, a Methods Evaluation Program was established within the Office of the Director to focus responsibility for the further development of this program. In November, 1970 a meeting of an advisory panel was held to consult on program substance and priorities and a solicitation for research resources was prepared. During December, 1970 two Request for Proposals were mailed to selected investigators relating to the following subjects: Evaluation of Blood Pressure in Oral Contraceptive Users (CPR 71-5) and the general topics of steroid metabolism and the biochemical pharmacology of steroid drugs (CPR 71-6). During January, 1971 the Fertility Regulating Methods Evaluation Branch was formally established and two professional and one clerical staff members were transferred from the Office of Director; one additional professional joined the staff in April, 1971. A review panel met during February, 1971 to review the responses to the Request for Proposals; approximately one-third of the proposals were approved for funding and these were negotiated during the conclusion of the fiscal year.

In these new programs, particular attention was given to the development of studies of the metabolism of representative steroid drugs, evaluation of relationships between drug dosage and various physiological effects, investigation of potential interactions between these drugs and various commonly used drugs and the mechanism of effect on blood pressure regulating systems. Additional epidemiological studies of thromboembolism and of linkage of in-hospital patient records were developed.

A variety of ongoing programs was under support during the year. The comprehensive multiphasic screening evaluation of contraceptive users at the Kaiser Foundation Hospital, Walnut Creek, Calif., was continued. The phase of active recruitment was completed and supportive computer systems and programming will be finalized shortly. Present plans call for an intensive effort to increase return visits by patients previously enrolled in the study and a schedule for analysis of selected items of data. Preliminary information on blood pressure and blood coagulation in the population has now become available.

A study of a precursor of cervical cancer as related to contraceptive use continued at the University of California at Los Angeles. Recruiting has continued and a preliminary report describing differences in the prevalence of this lesion as related to choice of contraceptive has been published. Other active studies include continuation of evaluation of the effects of intrauterine devices on a variety of endocrine parameters at the Agricultural Research Service in Beltsville, Md.; a prospective study of chromosomes in users of oral contraceptives will be entering its final year of observation; two case-control

studies to evaluate the relationship between use of ovarian hormones and carcinoma of the breast are now in the phase of active case recruitment, while a study to evaluate the relationship between cerebro-vascular disease and oral contraceptive drug use is now moving into its final period of case collection.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title	:	Health Effects of Oral Contraceptives: Prospective Cohort Study
Contractor	:	Harvard University, Cambridge, Mass.
Money Allocated	:	$60,000 (FY 1971 funds)

Objectives: The proposal outlines plans to secure definitive epidemiological data on the morbidity resulting from adverse effects of oral contraceptives. Studies are designed to provide information not only on diseases presently under consideration, but also on possible conditions which are not yet suspected to result from use of oral contraceptives.

Significance to Biomedical Research and Program of the Institute: The implementation of a broad based epidemiological surveillance program for a complete spectrum of adverse drug effects has been of basic scientific concern in the field of clinical pharmacology. It may provide the Center of Population Research with the methodology to continuously monitor the safety of contraceptive therapy.

Proposed Course: Data from all discharge records of married women from 39 hospitals in the Boston area will be linked to an existing data bank of 75,000 women previously established for studying the possible association of breast cancer with the use of oral contraceptives. Annual questionnaires will be utilized for implementing follow-up studies of the cohorts.

The work in the first year will be initiated in June 1971. It is primarily aimed to determine the feasibility and cast of the extended and elaborate surveillance procedures.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : The Study of the Relationship Between Thromboembolism and the Use of Oral Contraceptives
Contractor : The Johns Hopkins University, Baltimore, Md.
Money Allocated : $100,000 (FY 1971 funds)

Objectives: The purposes of this study are: 1) to investigate further the association between oral contraceptive use and thromboembolism in order either to substantiate or to define further the risks of developing these phenomena among users; 2) to provide a continuing surveillance of the risks of thromboembolic disease associated with the usage of oral contraceptives and to monitor adverse reactions to new formulations as they come into wider use;3) to define further any possible predisposing factors that may increase the risk of developing thromboembolic disease in oral contraceptive users; 4) to determine if there is a difference in the relative risk of developing thromboembolic disease associated with different formulations of oral contraceptives, e.g., sequentials and non-sequentials; and to determine if a dose-response relationship occurs with increasing amounts of estrogen; and 5) to investigate further the reported propotional lesser incidence of thromboembolism in persons of blood group O.

Significance to Biomedical Research and Program of the Institute: Several studies have now established an association between oral contraceptive usage and the development of thromboembolic disease. Questions as yet unanswered concern the relative risk of developing thromboembolic disease as related to usage of different formulations of oral contraceptives containing various amounts of estrogenic compound or a progesterone. In addition, it is still unclear which medical conditions or predisposing factors increase the risk of developing thromboembolic disease among oral contraceptive users.

Studies supported by this contract should provide essential information to establish or rule out potential contraindications to the use of oral contraceptives. It should also help to determine which formulations of these agents present the greatest hazards to users. This information may be essential to the Center for Population Research to carry out its assigned mission.

Proposed Course: The population to be studied will consist of women admitted to any of approximately 55 participating hospitals and diagnosed as having thromboembolic disease. It is planned to have 800 cases and controls under investigation over a three year period starting in June, 1971. For each subject data will be collected concerning the usage of oral contraceptives, demographic characteristics and medical history.

Statistical analyses appropriate to the design will be carried out to study differential usage of oral contraceptives in the case and control groups. Measures of relative risk of thromboembolism by formulation and by predisposing

factors will be obtained through chi-square and likelihood ratio techniques. Surveillance of risks on an on-going basis may be based on a sequential analysis of the data as it is collected.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title	:	Biochemical and Physiological Evaluation of an Oral Chemical Contraceptive Agent
Contractor	:	Baylor College of Medicine, Houston, Texas
Money Allocated	:	$50,000 (FY 1971 funds)

Objectives: Work to be supported by this contract should lead to a quantitative characterization of the absorption, metabolism and excretion of norethindrone.

Significance to Biomedical Research and Program of the Institute: Detailed knowledge of the metabolism of contraceptive drugs is necessary in order to understand the basic pharmacology of these agents and may influence their clinical application in fertility control.

Proposed Course: Analytical methodology will either be developed or refined in order to detect, quantify and identify a variety of steroids which are present in extremely small quantities in plasma and urine. Techniques to be developed will include: capillary and column chromatography and various types of mass spectrometry. The work on the development of methodology will be primarily applied to elucidate normal and possible abnormal metabolic pathways of contraceptive steroids, in particular that of norethindrone.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : A Study of the Metabolism and Effects of Steroidal Oral Contraceptives
Contractor : Medical College of Georgia, Augusta, Ga.
Money Allocated : $30,000 (FY 1971 funds)

Objectives: The purpose of the studies supported by this contract is to provide quantitative estimations of half-life, volume of distribution and metabolic clearance rates of synthetic estrogens and progestational agents in women with normal menstrual function. Drugs to be primarily investigated will be mestranol and norethindrone.

Significance to Biomedical Research and Program of the Institute: Detailed knowledge of the metabolism of contraceptive drugs is necessary in order to understand the basic pharmacology of these agents and may influence their clinical application in fertility control.

Proposed Course: The following protocol will be carried out in the conduct of the mestranol study: 1) First day, 100 micrograms of mestranol administered to three to five patients; 2) second day, 100 micrograms of mestranol repeated; plasma half-life and volume of distribution will be measured by injecting a priming dose of ^{3}H-mestranol and collection of numerous blood samples. Patients will then be infused with tritiated steroid at constant rate until a steady state is achieved. Urine will be collected for a period of 72 hours and a separation procedure done to determine the nature of the metabolites produced; and 3) patients will then be started on a sequential oral contraceptive preparation containing 80 micrograms of mestranol and 2 milligrams of norethindrone. Patients will be restudied after one to two months and after six to eight months of such medication to evaluate changes in half-life, volume of distribution and metabolic clearance rate.

For the norethindrone work, the following protocol will be adhered to: 1) Three to five patients will be given 10 milligrams of norethindrone on the first day; 2) on the second day, the dose will be repeated and plasma half-life and volume of distribution will be measured by injecting a priming dose of ^{3}H-norethindrone and collection of numerous blood samples. Patients will then be infused with tritiated steroid at constant rate until a steady state is achieved. Urine will be collected for a period of 72 hours and a separation procedure done to characterize the metabolites produced; and 3) patients will then be placed on continuous norethindrone therapy, 1 to 2 milligrams per day; they will be restudied after one to two months, and six to eight months of such therapy to evaluate changes in half-life, volume of distribution and metabolic clearance rate.

Separations between free urinary steroids with radioactive label and sulfate and glucuronide metabolites will be performed. Chromatographic separation

using paper and thin layer techniques will be done in order to study the nature and quantity of the various metabolites. Sulphates will be hydrolyzed by solvolysis and glucuronides hydrolyzed enzymatically by B-glucuronidase. Tritiated mestranol and norethindrone will be infused at a constant rate using a Harvard infusion pump and steady state established. Standard calculations of half-life, volume of distribution and metabolic clearance rate will be made.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Metabolism and Metabolic Effects of Steroidal Oral Contraceptives
Contractor : Worcester Foundation for Experimental Biology
Money Allocated : $170,000 (FY 1971 funds)

Objectives: Three research programs to be supported by this contract are aimed to: 1) define and identify metabolites of 17α-ethinyl estradiol, mestranol and norethinodrel in women, rats, hamsters and sheep; 2) measure the metabolic clearance, plasma half-life and rates of conversion of the three synthetic steroids; and 3) ascertain the effects of these agents upon tryptophan and serotonin metabolism.

Significance to Biomedical Research and Program of the Institute: Detailed information on metabolic transformations and pharmacokinetics of synthetic steroids will contribute to a better assessment of their medical effects, which is an important goal of the Center for Population Research. It will be of particular importance to get answers to the question as to what is happening to serotonin levels in women on long-term contraceptive medications. This is necessary before relationships between serotonin levels and clinical condition in the long term use of these drugs can be evaluated and, possibly, subjected to corrective therapy.

Proposed Course: Work under this contract will be initiated in June 1971. This is expected to lead to the identification of various urinary metabolites of three synthetic steroids, as well as to a characterization of their pharmacokinetic properties and their effect on tryptophan metabolism. Significant interim results for all three project areas supported by this contract should be available by June 1971.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Alteration of Thrombogenic Potential by Oral Contraceptive Steroids in Rhesus Monkeys
Contractor : Bionetics Research Laboratory, Div. of Litton Industries
Money Allocated : $65,000 (FY 1971 funds)

Objectives: The objective of this contract is to determine whether administration of contraceptive steroids to female rhesus monkeys leads to manifestations which might be related to the thromboembolic side effects encountered in patients on oral contraceptive drugs. In specific terms it will be attempted to demonstrate whether a combination of mestranol and norethindrone produces alterations in ultrastructure of vascular endothelium and platelets or whether it affects the humoral part of the coagulation process.

Significance to Biomedical Research and Program of the Institute: Thromboembolic disease in users of oral contraceptives remains the most serious documented side effect of these agents. Little is known of the pathophysiological mechanism which leads to this complication. An animal model would appear most desirable both for studying mechanisms and for developing techniques for prevention and therapy.

Proposed Course: A colony of 50 female monkeys will be treated with combinations of mestranol and norethindrone for seven menstrual cycles at human dose levels and 10 times human dose levels on the basis of body weight. Ultrastructure of platelets and endothelial cells will be studied by electromicroscopy before and at the end of treatment. A series of thirteen coagulation tests will be performed prior to initiation of therapy and once during each menstrual cycle. During the final cycle an *in vivo* test for thrombogenesis will be performed on mesenteric vessels and effects observed and recorded cinematographically. At the conclusion of the study the monkeys will be sacrificed and subjected to a complete detailed necropsy examination. All tissues, including a series of blood vessels will be subjected to histological examinations.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Studies of Dose-Response Relationships Between Low Dose Steroid Contraceptive Drugs and Plasma Gonadotropin Levels
Contractor : University of Rochester School of Medicine and Dentistry
Money Allocated : $20,000 (FY 1971 funds)

Objectives: The general purpose of this proposal to study systematically the plasma gonadotropin response to a number of combinations and dosages of these drugs with the expectation that one or more low-dose combinations can be found which consistently block ovulation with little or no effect on blood coagulation factors.

The specific primary aim of this proposal is to establish the lowest effective dose of several combinations of synthetic estrogens and progestogens which will reliably suppress the ovulatory surge in LH and FSH, and at the same time will be associated with either minimal or no measurable changes in blood coagulation factors II, VII, VIII and X, which are known to be elevated during oral contraceptive therapy. A secondary aim of this proposal is to identify which effective combinations have the least objectionable side effects of hyperestrogenism.

Significance to Biomedical Research and Program of the Institute: A number of studied have indicated the possible importance of minimizing the dosage or adjusting the proportion of estrogen and progestin in presently available contraceptive formulations. It is likely that at least some of the undersirable side effects of these agents might be minimized by reducing the dose of drug to a level which is associated with adequate efficacy but minimal undesired effects. The study will give useful insight into this matter at least for certain drugs and may indicate the eventual utility of this particular type of approach as a more general evaluation procedure.

Proposed Course: Approximately 30 normal college women, aged 18 - 25, will be studies for at least 6 months. Each woman will undergo a complete physical examination including pelvic exam and Pap smear which will be repeated every six months during the course of the study. Base-line estimations of plasma luteinizing hormone and follicle stimulating hormone as well as blood coagulation factors II, VII, VIII, X, euglobulin clot lysis and platelet aggregation will be determined during a normal control menstrual cycle prior to the administration of any ovulatory suppressant drugs. Specifically, during the control and subsequent menstrual cycle these determinations will be performed on days 12 - 18 of the cycle.

The drugs to be tested include ethinyl estradiol, mestranol and megestrol. At the beginning of the study, drugs will be administered in therapeutic dosage and then will subsequently be administered in progressively diminishing doses with determinations of plasma gonadotropins and blood coagulation factors as

described. The dosage schedule and combinations of drugs will be agreed upon mutually between the project officer and principal investigator.

After the lowest dose producing complete suppression of LH and FSH is determined for each combination of synthetic estrogen and progestogen, particular emphasis will be placed upon determining the reliability of this combination in blocking ovulation and repetitive cycles.

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Human Metabolism of Steroid Contraceptives
Contractor : University of Arizona
Money Allocated : $71,682 (FY 1971 funds)

Objectives: Studies are proposed to elucidate the metabolism of contraceptive steroids given both acutely and with chronic administration. It will be attempted to find metabolic correlates for a variety of clinical responses encountered in oral contraceptive therapy such as: Differences in patients with A, B, O blood groups and in those who have experienced thromboembolic complications. Further objectives are to determine the effects of other drugs on the metabolism of contraceptive steroids; to characterize their binding to specific uterine receptors; and to quantitate the relationship between contraceptive steroid therapy and plasma levels of endogenous steroid hormones.

Significance to Biomedical Research and Program of the Institute: The widespread use of synthetic female sex hormones in oral contraceptive programs has necessitated studies on the metabolism of these steroids in man. In spite of the extensive use of these estrogens and progestogens there is limited quantitative data on the metabolism of these steroids in humans. Whereas, information on urinary metabolites of plasma estrogens and progestogens is available, it does not necessarily reflect rates of their metabolism. Because formation of steroid metabolites and their subsequent excretion are separate and distinct processes, they do not necessarily occur in parallel. The biotransformation of estrogens and progestogens may result in the production of metabolites with altered biological activities, plasma protein affinities and renal and gastrointestinal clearances. Therefore, plasma levels of the parent steroid and its metabolites as well as the urinary metabolites are important in the assessment of the metabolism and the physiological (contraception) and toxicological (side effects) of these agents.

An objective of the Fertility Regulating Methods Evaluation Branch is to establish elements of increased risks and possible modification of the risk following acute and chronic administration of the steroid contraceptive drugs. The proposed study represents a comprehensive and sophisticated approach to the practical problems of drug use in these women of reproductive age.

Proposed Course: In June, 1971 studies will be initiated on the metabolism of some of the estrogens and progestogens used in oral contraceptive regimens. The steroids (ethinylestradiol, mestranol: chlormadinone norethinodrel, norethindrone) will be studied in healthy non-pregnant females of child-bearing age (18-34 years of age). The metabolism of the steroids will be studied both acutely and in patients on long-term therapy. Metabolism in these groups will be assessed after administration of labeled steroid by both oral and intravenous routes. The experimental plans encompass studies of: 1) subjects with A, B, O blood groups, and acute studies of subjects who have

experienced thromboembolic-thrombophlebitic episodes while on chronic oral contraceptive regimens; 2) the effects of other drugs (phenobarbital, diphenylhydantoin, benzodiazepines, aspirin, antihistamines, etc.) on the metabolism of the contraceptive steroids; 3) the effects of acute and chronic exposure to the contraceptive steroids on the endogenous plasma levels of estradiol, estrone, aldosterone, progesterone, testosterone; and 4) the binding of synthetic estrogens and progestogens to specific uterine receptors in humans in vivo and in vitro.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Five-Year Prospective Study of Abnormal Cervical Cytology
Contractor : Temple University, Institute for Survey Research
Money Allocated : $768, 790 (for six months)

Objectives: This five year prospective study is planned and designed to determine the relationship between abnormal cervical cytology and use of contraceptives. The main question to be answered is whether contraceptive drugs are involved as causal factors in the development of cervical cancer. A secondary objective is to determine whether such a relationship exists for the development of breast cancer.

Significance to Biomedical Research and Program of the Institute: A comprehensive, large scale, long term, prospective study for surveillance of the possible carcinogenic risks of oral contraceptive therapy has been urged on numerous occasions by a variety of scientific governmental advisory committees. The investigative work to be carried out under the contract represents a desirable implementation of these recommendations. The magnitude of expense, effort and difficulty must be recognized as a necessary concomitant of such an undertaking. The project is entirely relevant and essential to the mission of the Center for Population Research and its general objective of assessing the risk of contraceptive use and how they may be correlated with other individual factors.

Proposed Course: The study will be conducted in 10 collaborating clinics selected to provide a balanced distribution of contraceptive method, age, ethnic, and socioeconomic factors. A total population of 32,000 patients will be enrolled. They will be followed for five years with annual medical exmanination and Papanicolau smears.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Study of Differential Effects of Estrogen, Progestin as Contraceptive Agents
Contractor : New York University
Money Allocated : $68,648 (FY 1971 funds)

Objectives: This study is designed to determine the incidence, duration and severity of abnormalities of blood glucose, blood trigylcerides, prebeta lipoproteins, insulin and growth hormones which result from contraceptive therapy.

Significance to Biomedical Research and Program of the Institute: The metabolic and hormonal changes induced by by these agents are abnormal and may be potentially dangerous. For example, though it has not been established that hypertriglyceridemia causes arteriosclerosis,the evidence is convincing that hypertriglyceridemia, hypercholesterolemia and hyperinsulinism are closely associated with coronary artery disease (Albrink and others). Hyperlipemia is associated with abnormalities in clotting and coagulation, as well as with impaired glucose tolerance and with hyperinsulinism. It may be essential to avoid these effects, particularly in women who are predisposed and are easily unbalanced. The proposed studies are designed to detect these changes before permanent changes occur, to compare various compounds in the same woman, and to select and administer the proper agent to each woman. Certain women may be susceptible to one estrogen but not to another, e.g., mestranol and ethinyl estradiol. They may be susceptible to a progestin that is converted into substances having estrogenic activity, but not to one that is not, e.g., norethynodrel and chlormadinone acetate. Cross-over experiments will determine comparative effects in the same woman so that the proper contraceptive can be administered.

Proposed Course: Work to be supported by this contract is a continuation of a study of the effects of various commercially available oral contraceptives on a variety of parameters related to blood glucose and lipid levels in normal women, obese women, diabetic women who are being treated with insulin or oral hypoglycemic agents, and a group judged to be potentially diabetic on the basis of family or pregnancy history. Contraceptive preparations containing three different dosage levels of mestranol and two different dosage levels of ethinyl estradiol will be used. Following control tests, each women will receive one of these contraceptive agents (or an IUD during the control stage) for three to six months depending upon whether she develops any significant change in the lipid or glucose metabolism parameters measured. After discontinuing that particular compound and after having reached either base-line level, or after a period of one month, each woman would then be placed on a different compound which has been previously selected. All the compounds would be administered simultaneously and in random fashion to the group of women in order to minimize the effect of possible uncontrolled variables which might effect the population during the course of the study. Parameters to be measured include triglyceride, cholesterol, lipoproteins, glucose insulin and growth hormone measurements.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Oral Contraceptive Agents and Thromboembolic Complications
Contrator : Washington University School of Medicine, St. Louis, Mo.
Money Allocated : $94,000 (FY 1971 funds)

Objectives: The objectives of this contract are: 1) to develop, improve and standardize a new diagnostic method of fibrinogen chromatography; 2) to demonstrate the clinical usefulness of fibrinogen chromatography as a screening procedure for assessing the risk of thromboembolic complications in patients on various types of contraceptive therapy; employ the method of fibrinogen chromatography in a clinical trial of an antithrombolic agent (prostaglandin, PEG_1).

Significance to Biomedical Research and Program of the Institute: The development of a laboratory test capable to detect or predict intravascular coagulation is urgently needed in several fields of clinical and investigative medicine. It is of particular importance in contraceptive management, since unexpected thromboembolism appears to be a major well-documented side effect of contraceptive steroids.

Proposed Work: In addition to methodological and biochemical studies to improve and establish the usefulness of fibrinogen chromatography, the following clinical investigations will be carried out: 1) a cooperative study is to be undertaken with the Searle Company in which findings will be ascertained on women receiving an oral contraceptive, and one administered by an indwelling vaginal ring, and these will be compared to women on intra-uterine devices and those using diaphragms; 2) blood studies will be continued on women who are on long term oral contraceptive medication including some who have been taking this treatment for 10 years. Additional studies will be performed on post-menopausal women with and without estrogen treatment; and 3) a cooperative study will be undertaken with Dr. John O'Brien from Portsmouth, England, in which the Fletcher test will be compared to results obtained with 125-labeled fibrinogen scanning over the lower extremities in post operative patients.

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Effect of Oral Contraceptive and Conjugated Estrogen on Renin Activity and Aldosterone
Contractor : Loma Linda University, Loma Linda, Calif.
Money Allocated : $64,000 (FY 1971 funds)

Objectives: It will be attempted to clarify the pathophysiological mechanisms of steroid induced hypertension. Based on a better understanding of this condition, various methods of therapy will be explored in order to reduce elevated blood pressure caused by contraceptive drugs.

Significance to Biomedical Research and Program of the Institute: The scientific value of the proposed work lies in its promise to clarify pathophysiological relationships between steroid metabolism, electrolyte balance and hypertension. This knowledge is relevant to the program of the Center in order to assess risk and consequences of contraceptive induced hypertension and possibly devise an approach to its therapy and/or prevention.

Proposed Course: Work on this project will be initiated in June, 1971. The effects of contraceptive agents on the following parameters will be measured: Levels of plasma renin, plasma aldosterone, excretion of aldosterone and exchangeable sodium. Therapeutic trials will be conducted to assess the efficacy of spironolactone and derivatives in reducing contraceptive induced hypertension.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Medical Effects of Steroid Contraceptives: Prospective Studies of Carbohydrate and Lipid Metabolism and Blood Pressure
Contractor : University of Miami School of Medicine
Money Allocated : $84,000 (FY 1971 funds)

Objectives: The objective of this contract is to establish the incidence of abnormalities in carbohydrate and lipid metabolism resulting from long term use of contraceptives. Further aims are to determine the reversibility of these changes and possibly to identify high risk groups of patients susceptible to these adverse metabolic effects of a variety of contraceptive preparations.

Significance to Biomedical Research and Program of the Institute: The study is a valuable continuation of an ongoing extensive evaluation of the metabolic consequences and side effects of contraceptive agents. It has the special advantage of monitoring for these effects over a long period of time in the context of a well designed prospective study. An outstanding feature is the prescence of established facilities and procedures and a trained technical staff who has worked with the principal investigator for many years. For these reasons the project is an important element in the Center's program of continued surveillance of contraceptive therapy.

Proposed Course: Women who are more than four weeks postpartum or who have been off steroid contraceptives more than three months and who desire to use a method of family planning will be tested with a three hour oral glucose tolerance test before receiving treatment. Their medical history, weight and blood pressure will also be obtained. The blood will be analyzed for its glucose, insulin, growth hormone, and triglyceride content.

An available steroid contraceptive of combination, sequential or progestin-alone type will be selected for those who choose steroid contraception, and mechanical devices will be supplied to those who choose that method of contraception. A further group of women of comparable age who have had a hysterectomy, but who have remaining ovarian tissue will be given one of several estrogens after a similar testing procedure. All the women enrolled will be requested to return for repeat testing at six-month intervals. Re-testing of the women after they discontinue a contraceptive method will be performed. Each sub-group will have an initial enrollment of 150 to 200 patients. All of the historical physical laboratory data will be placed on punch cards for computer analysis. Duplicate copies of the punch cards and computer print-out will be sent to the project officer. Data analysis in the form of a narrative manuscript with tables and figures will be submitted as each study is completed. Progress reports will be submitted quarterly. These studies may: 1) allow assessment of the extent and magnitude of any risks of serious changes in the factors studied; 2) determine what types of steroids may cause these changes;

3) determine what types of women are most likely to experience the changes; 4) determine the reversibility of any alteration; and 5) possibly establish the order of metabolic change which may suggest the underlying mechanisms of etiology.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Physiological Mechanism Involved in Reactions to Implanted Devices in Animal Uteri
Contractor : Agricultural Research Service
U. S. Department of Agriculture
Money Allocated : $72,808 (FY 1971 funds)

Objectives: Work will be aimed toward elucidating the endocrine relationships, the mediators and the physiological pathways by which intrauterine devices, ovarian hormones, synthetic hormones, steroid hormone antagonists and prostaglandins affect the productive organs and the reproductive process.

Physiological responses of a variety of animals (cow, ewe, gilt, rabbits) to the presence of intrauterine devices will be investigated and the mechanism of action by which intrauterine devices interfere with normal reproduction will be characterized.

Specific problems under investigation include: (1) Elucidation of the mechanism(s) underlying the IUD-activated luteolysis,(2) assessment of the effects of IUD's on uterine contractility and ovum transport; (3) factors such as phagocytosis, contributing to the spermicidal effects of IUD's will be evaluated; (4) changes in ultrastructure and chemical composition of uterine tissue will be analyzed; (5) endocrinological effects of intrauterine devices on utero-pituitary-ovarian relationships will be documented; and (6) antiserum against various steroids will be produced.

Major Findings and Conclusions: The point at which intrauterine devices interfere with reproduction vary from inhibition of ovum fertilization in sheep to embryonic mortality in gilts. Intrauterine devices affect not only fertility and various aspects of uterine function, but extrauterine tissues as well. Alteration of pituitary gland function has been demonstrated in the rabbit. The development and maintainance of corpora lutea were found to be inhibited in sheep, cattle and guinea pigs. Among the more important findings with regard to the effects of an IUD have been the following: Inhibition of sperm transport and ovum fertilization in sheep; prevention of embryo survival *in utero* in cattle and swine; inhibition of corpus luteum formation and initiation of premature corpus luteum regression in sheep, cattle and guinea pigs; the operation of the IUD-activated luteolytic mechanism through a local utero-ovarian pathway; delay of pituitary gland release of luteinizing hormone and ovulation in rabbits; elevated vascular function in the endometrium of sheep and lowered vascular function in the endometrium of rabbits; increased uterine motility and reversal in the direction of uterine contractions in sheep; intensified leukocytic responses of the uterus to induced bacterial infection and increased bactericidal capabilities of the uterus in sheep and rabbits; increased spermicidal capabilities of the uterus in sheep and rabbits; altered chemical composition of endometrial ground substance in cattle, sheep and rabbits; increased variability in the sexual behavior of sheep.

the wide variations in the manifested effects of intrauterine devices do not necessarily signify differences in the basic physiological responses. Rather, these varied effects probably represent the consequences of a few primary responses through which animals attempt to rid themselves of foreign objects in their uterine cavity.

Significance to Biomedical Research and Program of the Institute: In spite of the widespread use of intrauterine devices, knowledge is incomplete as to the mechanisms by which conception is prevented. Still less is known concerning medical effects of these devices on other processes and organ systems. This study promises to fill some of these gaps in productive physiology and may also contribute towards the medical evaluation of this type of contraceptive therapy. Leads to the development of new contraceptive methods may also emerge from this productive investigation.

Proposed Course: The Institute has been supporting this research by reimbursable agreement with the Department of Agriculture since 1965. The work conducted has led to a major contribution to the available knowledge concerning the physiological responses of animals to intrauterine devices and, directly, to basic reproductive physiology.

It can be expected that the project will continue to supply necessary experimentation and new ideas to broaden our understanding of reproductive physiology and contraceptive management.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Chromosomal Breakage in Women Taking Oral Contraceptives
Contractor : AEC/Oak Ridge Associated Universities, Inc.
Money Allocated : $60,612 (FY 1971 funds)

Objectives: The purpose of this reimbursable agreement is (1) to confirm and extend earlier studies which suggested that use of oral contraceptives leads to an increased frequency of chromosomal breaks in cultured lymphocytes; (2) to determine whether the increased chromosome breakages observed in lymphocyte cultures from women taking oral contraceptives are representative of chromosome damage in other somatic cells.

Major Findings: Chromosome analyses from 10,000 metaphases from control men, 12,173 metaphases from control women and 14,313 metaphases from women taking oral contraceptives, have been computed. The accumulation of this large number of observations has disclosed patterns in chromosomal breakage that could not have been observed otherwise. The following trends have been defined: (1) Breakage frequencies are lowest in cultures from control men. (2) Breakage frequencies in women taking oral contraceptives are slightly, but significantly higher than breakages in control women. (3) The number of breaks per culture varies in different preparations from the same person. (4) The average breakage in each group are highest during the late spring and summer. (5) The distribution of breaks in different chromosome groups is non-random.

Significance to Biomedical Research and Program of the Institute: The study of chromosomal breakage is important for two primary reasons. (1) There is some evidence that suggests a correlation between chromosomal aberrations and malignancy. Such observations are frequently found in cells from many solid tumors as well as in malignant cells from patients with leukemia. In addition, it is well known that many carcinogens induce chromosomal breakages. (2) It is possible that those agents that damage somatic cells could in turn cause chromosomal aberrations in gametes, and potentially increase the probability of birth defects.

Although the full biological significance of chromosomal breakage is not understood, it is important to evaluate this effect of oral contraceptives to which large numbers of persons are exposed.

Proposed Course: This contract was effective July 1, 1968 and planned for three years. During the coming 3rd year, the studies demanded by the initial work scope will be completed. In addition, however, the work has been expanded to assess in more detail the significance of the increased chromosomal breakage previously demonstrated.

Specifically, the questions of whether chromosomal breakage by oral contraceptives is limited to lymphocytes or whether the relevant findings from these

cultures may be extrapolated to other somatic cells and possibly gametes will be considered. This will be accomplished by simultaneously culturing and examing blood lymphocyctes and skin fibroblasts from the same subjects.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Study of the Metabolic Effects of Oral Contraceptives
Contractor : University of London
Money Allocated : $14,808 (FY 71 funds)

Objectives: The major purpose of this contract has been to investigate the metabolic effects of oral contraceptives in a long term prospective study. Special focus was on changes occurring with long-time use of oral contraceptives in the transformations of carbohydrates, lipids, tryptophan and Vitamin B6.

Summary: This has been a highly productive contract. More than 1,000 subjects have been studied in a prospective manner. The results of this work have appeared in numerous articles and several monographs. It has greatly contributed to our present awareness of the metabolic consequences of oral contraceptive therapy and indicated the need for careful surveillance programs to assess the clinical signifiance of the contraceptive-induced alterations of carbohydrate and lipid metabolism.

Significance to Biomedical Research and Program of the Institute: Long term prospective studies were required in a large number of patients to identify the metabolic consequences of oral contraceptive therapy. At the time of initiation of this contract in 1967 the project was considered to be unique and American investigators had not yet developed a comprehensive research program of sufficient magnitude to accomplish the large scale prospective surveillance of contraceptive users for adverse metabolic changes.

Proposed Course: This successful contract is being terminated since research programs have been developed within American institutions with capabilities to pursue this type of work and assess the clinical significance of past results.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Contraceptive Drug Study
Contractor : Kaiser Foundation Research Institute
Money Allocated : $1,059,995 (FY 1971 funds)

Objectives: The objective of this contract is to evaluate the health effects of oral contraceptives in women who are members of the Kaiser Permanente Medical Insurance Plan at Walnut Creek, California. The principal tool for accomplishing this objective is an automated multiphasic examination with which up to 12,000 women will be studied annually. The facility is also expected to develop the capability of studying new contraceptives as they are developed, and to introduce into the examination schedule test procedures that are of current interest.

Summary: The objectives and techniques of this project are still considered to be an important goal of high priority. The study has shown that a cohort of patients of suitable size and distribution can be assembled and subjected to the diagnostic procedures necessary for evaluating the medical effects of oral contraceptive drugs. Emphasis will now be placed on obtaining the necessary follow-up examinations and analysis and evaluation of the expected prospective data. Although there have been unfortunate delays in data processing, data analysis and manuscript preparation, the schedule for operations has improved.

Significance to Biomedical Research and Program of the Institute: Long term prospective studies are required to identify possible adverse effects of contraceptive agents presently in use or of others which may be developed. This study is an important part of the Center's program to monitor the medical effects of oral contraceptives.

Proposed Course: Progress of patient recruitment and follow-up, data processing, analysis and manuscript preparation will be closely monitored by NICHD staff and consultants. It is hoped that these measures will bring about the timely realization of the objectives which are of potentially great value to the program of the Center for Population Research.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Effects of Steroid Contraception on Cervical Dysplasia
Contractor : University of California, Los Angeles
Money Allocated : $ 32,328 (FY 1971 funds, est.)

Objectives: The contract is to support the study of the cumulative effects of steroid contraception on the occurrence of cervical cancer by studying dysplasia of the cervix. This program has been in operation since June 1967. Patients from family planning clinics of the Los Angeles County Health Department are entered into the project for a prospective study on the relationship between progression of cervical dysplasia and the use of oral contraceptives.

During the first two years of operation, the project was centered in the inner city where the racial composition of the target population and, consequently, of patients entering the study was predominantly black (82% of all eligible patients). The expansion of the project during the last year into East Los Angeles, Hollywood-Wilshire, Whittier West and Northeast, has led to changes in the demographic and socio-economic characteristics of the population under investigation. It now includes a larger proportion of Spanish Americans and Caucasians in higher income levels than the original populations. The aim of enrolling at least 200 patients in study and control groups has been achieved.

Major Findings: As a first step to evaluate a possible differential in carcinogenic development between pill users and controls, the characteristics of pill choosers and their controls (IUD choosers) were investigated. Women choosing the pill differed from those choosing IUD's in having higher income and lower body weight, but no further differences were found over a wide range of demographic and biomedical characteristics, including religion, ethnic group, age at first intercourse, number of children, etc.

The major finding of the program so far has been published in Science, 31 July 1970, Vol. 169, pages 497-498: A significant association of dysplasia was found with choice of the contraceptive pill compared to choice of IUD, or of the IUD and other methods. These findings have been updated and extended from a predominantly black population of contraceptive users to other ethnic groups. The generalization of an increased prevalence of dysplasia associated with pill choosers seems to hold for all three ethnic groups examined; black, Caucasian and Spanish. Experience is still too limited to assess whether there is a differential incidence rate in development of dysplasia between drug users and IUD users or whether oral contraceptives affect the transition from dysplasia to malignancy. While the project has provided a fertile field for the study of human behavior in a follow-up program, the follow-up study of cervical pathology has had many difficulties.

Significance to Biomedical Research and Program of the Institute: This proposed work, if carried out successfully, could be of great value in assessing the

risks of contraceptive therapy and is, therefore, clearly relevant to the program of the Center.

Proposed Course: The follow-up studies of patients on various forms of contraceptive therapy will be continued. Emphasis will be placed on follow-up of patients already enrolled in the study. Data obtained will be systematically accummulated, analyzed, and should lead to the preparation of one or more definitive manuscripts.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : A Retrospective Study of the Risks for Cancers of the Breast, Body of the Uterus, Ovary, and Cervix Among Users of Oral Contraceptives.
Contractor : Yale University, New Haven, Connecticut
Money Allocated : No funds allocated for FY 1971

Objectives: To determine whether the incidence of carcinoma of selected sites is associated with oral contraceptive use.

Summary: Cases are being identified of women with breast, uterine, and ovarian cancer admitted to hospitals in the areas in and around Bridgeport, Hartford, and New Haven, Connecticut, and Springfield, Massachusetts, over a two-year period. A total of 900 such cases is expected between the ages of 20-44.

Controls are selected as the next patient with the appropriate matching variables admitted to the same hospital as the case with an acute medical or surgical condition or for an elective operation.

Interviews are administered in hospital, whenever possible, inquiring of demographic variables, obstetric history, menstrual history, contraceptive history, and a checklist of diseases.

Analysis will involve an examination of the difference in the proportions of cases and controls exposed to oral contraceptives for different lengths of time, for each site and for specific cell-types at each of the four sites. Relative risks for various lengths of exposure to the oral contraceptive will be calculated.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: In the initial year, reporting mechanisms, matching procedures, and questionnaires have been developed. A considerable number of cases have been identified and interviewed. The coming year should provide a sufficient number such that an analysis is completed, a decision will be made as to whether this surveillance system needs to be continued, and if so, at what case finding level.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Oral Contraceptives and Tumors of the Breast
Contractor : University of California School of Public Health
Money Allocated : $74,506

Objectives: To determine whether the incidence of tumors of the breast is associated with oral contraceptive use.

Summary: The frequency of oral contraceptive use in women with breast cancer and women with benign breast lesions will be compared with that of control groups of women free of cancer and/or breast lesions. Cases diagnosed in a two-year period will be selected.

Three groups of women are being used:

1) Women with breast cancer 49 years of age or younger in whom cancer of the breast has been diagnosed for the first time during the study period. These are patients admitted consecutively to certain designated hospitals in San Francisco Bay Area.

2) Women with benign breast lesions. These are women biopsied or operated for benign lesions of the breast in the same hospitals and within a specified period of the cancer cases. One woman with a benign breast lesion is matched with each cancer by age, by race, by religion, and by socio-economic status.

3) Women without evidence of breast lesions or cancer of any site. These women serve as controls. Two controls are chosen for each cancer case, one from surgical and one from the medical service.

The aim is to accumulate at least 400 cases in each of the study groups, women with breast cancer, with benign breast lesions, medical controls and surgical controls.

Basic data necessary for the study are derived from two main sources--review of the hospital record of each subject and a personal interview. The interview seeks information on socio-demographic, menstrual and reproductive and contraceptive variables.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: In the initial year, reporting mechanisms, matching procedures, and questionnaires have been developed. A considerable number of cases have been identified and interviewed. The coming year should provide a sufficient number such that an analysis of these data can be made. When such an analysis is completed, a decision will be made as whether the surveillance system needs to be continued, and if so, at what case finding level.

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Center for Population Research
Fertility Regulationg Methods Evaluation Branch

Contract Title : A Collaborative Study of Oral Contraception and Cerebrovascular Disease
Contractor : Duke University Medical Center
Money Allocated : $50,358

Objectives: To determine whether the incidence of stroke in young women in increased by use of oral contraceptives, and if so to identify characteristics that are especially related.

Summary: During a two-year period the Neurologic Services of ten university medical centers have identified all young women admitted to their hospitals with definite or suspected cerebrovascular disease of any type. Four of the participating hospitals also identify patients admitted to each of the community hospitals in their metropolitan areas (Miami, St. Louis, Baltimore, and Atlanta). An estimated 200 patients with cerebrovascular disease are submitted for study each year. Clinical data on each of these patients are abstracted from the hospital and physician's records. The frequency of usage of oral contraception in these patients will be compared with that found in two selected control samples: 1) women hospitalized for other diseases and 2) women representing a "healthy" population residing in the same neighborhood as the patients selected for the study. Information regarding pill usage will be obtained from the patient and control subjects by personal interviews carried out in their homes.

Almost all patients and controls have been selected and interviewed in this year. All clinical data have been assembled at Johns Hopkins, where a coding system has been developed. The final year will be spent in data processing, analysis, and development of a report of the study.

Significance to Biomedical Research and Program of the Institute: The study is an extension of previous research on thromboembolism and oral contraceptive use in which very few stroke cases were included and is a part of our program to monitor the significant effects on health of currently used contraceptives.

Proposed Course: All aspects of the study should be completed by the summer of 1972.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title	:	Study of Correlates of Intrauterine Contraceptive Device Effectiveness
Contractor	:	University of California, Berkeley School of Public Health
Money Allocated	:	Extended without further funds

Objectives: The aim of the study is to search for physiological, attitudinal and demographic factors which are associated with effectiveness and acceptability of the IUD. The approach is to obtain information through standardized procedures on women provided with IUDs at public family planning clinics throughout California.

Summary: Dr. Albert Pierce was appointed as the new project director for this study in September of 1969. Dr. Pierce is on leave of absence from San Fernando Valley State College, where he is Professor of Sociology. He will be with the study through the final funding year. His task is to complete the last phase of the study, closing out of the data collection, analysis and reporting of the results. The data consist of information collected on approximately 9,000 women provided with IUDs in 11 county health department and hospital clinics throughout California.

Significance to Biomedical Research and Program of the Institute: This is an area of increasing importance as IUDs are increasingly looked to as an alternative to oral contraceptives.

Proposed Course: The study has been extended without further funding. A final report is being prepared for publication which will be available this coming year.

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Behavioral Sciences Branch
Center for Population Research

Research relating to population examines the interrelationships between population size, growth and distribution and economic development and other social and psychological factors and analyzes ways in which individuals are motivated to reduce the size of their families. It is, therefore, concerned with a great variety of processes. It takes into account individual, interactional, familial, institutional, and social processes of great complexity involved in decisions relating to family size and the effective implementation of population policies and their consequences. Research in this area has been greatly expanded in line with NICHD's designation as the focal point for DHEW research in population.

Ad hoc advisory panels were established in FY 69 to guide the development of a program of research and to evaluate research proposals which are developed in the behavioral sciences in four areas:

(1) The first of these areas of population research is concerned with the antecedents, processes and consequences of population structure, distribution, and change, and the interrelationships between social and population change. Special importance is attached to the interrelationships between economic factors and population growth, structure and distribution for this and other nations and for particular subgroups of the nation. Of increasing concern is the ecological impact of population growth and of related urban technological and industrial development to guide the formulation of policies with reference to environmental quality. Attention is also being given to migration as a major vehicle of social change.

(2) Major concern focuses on trends in fertility and related variables, such as marriage rates, age at marriage, unintended pregnancies, the incidence of abortion, trends in divorce, and changes in attitudes toward childbearing, family size, methods of fertility control, and other topics, including the failure to prevent unwanted births despite verbal commitment to family planning. Considerable effort is being made to develop a broader data base for more rapid determination of trends and the factors affecting them.

(3) Ultimately the factors affecting trends in fertility will express themselves mainly within the framework of the family, so that a major area of research on population must concentrate on the family, on differences in this institution among various population groups, and on

changes which are occurring in its structure, particularly as they affect fertility. Emphasis must also be placed on sexual behavior, both in and out of marriage, insofar as this bears on fertility. Research is also sought on the process of socialization for marriage and parenthood, including the social pressures exerted by the immediate family, kin networks, peers, and the broader society to have--or refrain from having--children. More needs to be known about the factors influencing decisions that lead to the number and spacing of births, and including perceptions of alternatives to childbearing. Needed, too, is research on the relationship between family structure, childbearing patterns, and child development.

(4) Concern with problems of population growth leads to questions of public policy. Research on all aspects of growth is thus relevant to policy but is also needed specifically on the ways by which population growth or movement are influenced by public policy, and the effects upon population of policies already adopted regarding family planning programs, welfare payments, parity payments, tax exemptions and allowances, direct incentives, etc. As to the relationship with family planning programs, the Center for Population Research is concerned with research on the assumptions under which programs are organized and the evaluation of these programs in terms of those assumptions; operational research is considered the primary responsibility of other agencies.

About one-in-five of the proposals submitted under the contract program in the behavioral and social sciences have been approved by peer review committees and funded. Funding of approved projects totalled $0.7 million in fiscal 1969, doubled to almost $1.6 million in fiscal 1970 and redoubled to approximately $3.5 million in fiscal 1971. Since the initiation of this research program in early 1969, a total of 52 contracts have been funded. Investigators in a wide variety of institutions, mainly universities, but also in governmental and private research organizations, have become involved in the research program. Fields of specialization represented are sociology, psychology, economics, anthropology, medicine, and statistics.

It is too early in this phase of the program to report definitive results, as only a few studies have been completed. Most are still in the active stage, but findings from several are expected in the next several months. Others will not be completed for another year or two. As those now underway and others to be initiated are completed, however, it is hoped that our knowledge of the influences upon the birth rate and completed family size will be greatly expanded.

Much of the discussion about "the population problem" assumes that population plays a large role in many of our other social problems, but to what extent, and in what ways, are not sufficiently well known or agreed upon by

competent scholars. Better knowledge concerning the effects of population growth, structure, and distribution are essential if informed and acceptable public policies regarding population are to be formulated and adopted. These studies, dealing with a variety of interrelated factors not easily disentangled, require that a program be structured in long-range terms, and it will be at least several years before results highly useful to policy makers will emerge.

Emphasis here is on projects which were continued (including those for which a final report was received) or funded and initiated in fiscal 1971. Currently there are 45 funded contracts (including six reimbursable agreements with other Federal agencies), 10 of which are extensions (with additional funds) of projects negotiated in previous years, and 12 of which are extensions without additional funds.

Fertility, Abortion, Illegitimacy and Family Planning

Center for Population Research contracts indicate both the concentation of interest in fertility and family planning and the range of problems dealt with. Under a reimbursable agreement with the National Center for Health Statistics, a study of measures of unwanted childbearing is being conducted by the Institute for Social Research, Temple University (DC-8).*

A study being done at the University of Michigan (DC-9) involves the analysis of data on child spacing and family planning in Malaysia, including a study of opinion leaders and the communication process related to family planning information and values. Researchers at the University of North Carolina (DC-10) are seeking to ascertain types of misunderstandings in communication between instructors and students in sex education classes in order to improve the quality of instruction in this area.

A contract with the Population Council (DC-11) concerns the dimensions of the abortion problem in the United States. The Center for Disease Control (DC-12) in Atlanta, as part of a reimbursable agreement to provide training and experience of physicians in the epidemiology of fertility regulation, has started an "abortion surveillance" program patterned after earlier and successful efforts to collect, evaluate, and distribute information on communicable diseases. A Temple University study (DC-13) is designed to assess physicians' current perceptions, understandings, and attitudes toward birth control methods which may be considered as possible abortifacients. The psychosocial components of repeated abortion seeking behavior of women in Hungary are being investigated by the American Institutes for Research of Silver Spring, Maryland (DC-14). A study on the consequences of illegitimacy, and methodological problems associated with studying the phenomenon, has been instituted at the University of California (DC-15).

An essentially methodological study, arranged under contract with the University of North Carolina (DC-16), seeks to ascertain through computer simulation the effects on fertility of various child-spacing practices in an

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* Number of page on which greater detail is given in the summaries of individual projects that follow this general summary.

effort to improve the analysis of current trends in fertility. A University of Pennsylvania (DC-17) project is designed to specify procedures for reducing the cost and complexity of dealing with the 1970 census tapes produced by the Bureau of the Census. This project should have broad applicability across the range of population subjects investigated in the behavioral sciences program of the Center for Population Research but will have specific applicability in permitting a focus on persons rather than on census tracts in local area research on fertility.

Socio-economic Correlates of Fertility

Eight studies deal with socio-economic correlates of fertility in various historical and cultural settings. Analysis of 1967 Survey of Economic Opportunity data dealing with the social and economic variations in marriage and fertility histories was completed in fiscal 1971 under a reimbursable agreement with the Bureau of the Census (DC-18). Rand Corporation (DC-19) research is attempting to identify the principal determinants--e.g., income, time of marriage, female labor force participation, and migration characteristics--of family size and current fertility patterns in the United States. The influence of socio-economic factors on fertility should provide the basis for estimating the probable impact on the birth rate of: (1) increased years of schooling, (2) unearned income supplements to the working poor, (3) change in tax deductions for dependents, (4) housing and rent subsidies, and so forth.

A contract with Princeton University (DC-20), a continuation of a study underway for several years, deals with the correlates of fertility reduction in European countries in the last two centuries. Another contract, with Ohio State University (DC-21), is studying demographic changes in Imperial Russia and the U.S.S.R., with particular emphasis on the effect of economic development and public policy upon fertility. The objective of research being undertaken at Western Washington State College (DC-22) is an investigation of social, political, and economic influences bearing on fertility, including rural differentials by size of land holding and abortion and fertility differentials by employment status of women in Poland, and the socio-economic correlates of fertility and birth control in the U.S.S.R.

A contract with the Food Research Institute, Stanford University (DC-23), is investigating the socio-economic correlates of fertility reduction in developing countries. Another study in this area, through a contract with Brown University (DC-24), deals with the socio-economic correlates of fertility as reflected in the 1960 and 1970 censuses of Thailand. Peasant communities in Indonesia, India, and Mexico and a farming community in the United States will be studied in the field by Columbia University (DC-25) investigators to assess the costs and benefits associated with different family sizes in each community.

Sex Roles and Socio-Psychological Correlates of Fertility

Other studies directly related to fertility approach its analysis from several different standpoints. The 1970 Survey of Fertility, being conducted by Princeton University (DC-26), will analyze fertility behavior and fertility

intentions of American women in terms of a variety of social and economic characteristics. In addition, several studies investigate the relation of fertility to marriage and family structure. A contract with Indiana University (DC-27) studies this relationship in terms of the definition of sex roles, emphasizing particularly the effect of employment of women. A study by the Institute for Juvenile Research in Chicago (DC-28) is exploring the changes during the freshman year in college in the self identity of girls in relation to balancing work involvement and social-sexual involvement.

A study, based on a complex model of the relationship between fertility and women's labor force activity being initiated in Research Triangle Institute (DC-29), N.C., suggests that antecedent social and economic variables determine labor force participation and fertility and that labor force participation affects fertility only indirectly. Based on data collected in the Greater Athens Metropolitan Area in 1966-67, a Wayne State University (DC-30) study is examining the antecedent factors relating the employment of women to fertility and birth control to explain how the level of urban Greek fertility can be low in the absence of (a) organized family planning and other governmental programs, and (b) economic industrial development.

Another contract, with the University of Wisconsin (DC-31), analyzes trends in marriage, divorce, remarriage, and their relationships to size of family, using data from the Survey of Economic Opportunity in 1967. Research underway at Bowling Green State University (DC-32) involves a re-interview of mothers interviewed in Toledo in 1963 coupled with a sample of married women in the Toledo SMSA to test for possible associations, within broad religious and socio-economic categories, between selected social-psychological and demographic variables. Under a reimbursable agreement with the Census Bureau (DC-33), a study of the relationship between age at marriage, birth intervals, and total fertility is being made based on a Current Population Survey.

A University of Massachusetts (DC-34) study will utilize data collected in 1964 under the guidance of the United Nations Demographic Center (CELADE) in Santiago, Chile to analyze mating and marriage patterns in seven Latin American capital cities as a determinant of fertility. A study being undertaken by Boston College (DC-35) is investigating the differences, over a wide range of variables, between large and small families in Puerto Rico.

Demographic and Attitudinal Factors Affecting Family Formation

A theoretical study is being made at the University of California (DC-36) of the changes in population structure which may arise from shifts in sex ratios at different ages and the effect of such changes upon age at marriage. Research underway in Massachusetts by the Worcester Foundation for Experimental Biology (DC-37) is testing attitudinal factors affecting family size. Other research, being undertaken in Louisville, Kentucky by the Human Resources Research Organization (DC-38), is exploring the factors involved in "moral judgment" with respect to marital status and family size. This study assumes that moral judgments are key factors in social pressures to conform, that single people and childless couples are widely viewed as blameworthy, and that this is deleterious to population control efforts.

Another study, being undertaken by Harvard University (DC-39), looks to the kin network in the United States as the locus of influence for its members with respect to fertility and migration decisions. A University of Kansas (DC-40) study is questioning a group of first-time parents to find factors--"purpose-rational" goal oriented, traditional, or economic--that are affecting their decisionmaking as to the number and spacing of births.

Migration, Social Mobility, and Residential Patterns

Eleven contracts deal with migration. One study of migration differentials in the United States, concentrating on differences among whites and Negroes by education and other social characteristics, is being undertaken at the University of Massachusetts (DC-41). A University of Georgia (DC-42) study analyzing the trends and patterns of population change for the total white and nonwhite populations of all counties in the United States from 1950 to 1960 is concentrating on fertility and migration. Another attempt to study the relationship between migration and fertility is being undertaken at Louisiana State University (DC-43).

A Brown University (DC-44) study, based on a probability sample of women residing in Aberdeen, Scotland, who had at least one live birth during the period October 1950-September 1955, is designed to investigate: (1) how spacing of pregnancy and of fertility varies; (2) the life conditions under which women are likely to resort to terminations and sterilizations; and (3) how the size of the family of origin and the position on a traditionalism scale are related to social mobility, present socio-economic status, aspiration for children, and fertility. Upward social mobility, given the economic means and a lack of social barriers, frequently has meant in the United States and Canada a change to different housing in another neighborhood--and perhaps in another city--for, among other purposes, childbearing and childrearing.

The U.S. Bureau of the Census (DC-45), under a reimbursable agreement, will combine data from the March Current Population Survey taken by the Bureau for the years 1966-1970 to study migration during that period by single years of age in order to assess the joint influence of various socioeconomic and familial statuses on the probability of making different types of moves during an individual's lifetime if he moved according to present age-specific rates of residential mobility. In another approach to migration research, under a reimbursable agreement with the U.S. Department of Commerce (DC-46), data from the one-percent sample of reports on the Social Security covered workforce are being tabulated to provide a data bank from which other investigators can study mobility among the States, SMSAs, and nonmetropolitan areas of the United States. These tabulations will measure inmigration, outmigration, and the nonmigrant workforce of each relevant area, cross-classified by age, sex, race, area of origin or of destination, industry of employment, and level of earnings.

A University of Michigan (DC-47) study is analyzing 1970 U.S. census data on tapes for 20-25 SMSAs (Standard Metropolitan Statistical Areas) and comparing these with census materials for four Canadian metropolitan areas to determine:

(a) variations in population distribution in different metropolitan areas; (b) the degree of segregation of families in different stages of the life cycle; (c) the degree of residential segregation of different socio-economic groups; (d) the pattern of segregation of housing of different types and ages; and (e) the relation of housing type to socio-economic characteristics of occupants.

Another study deals with the economic costs and benefits of migration to areas which lose and areas which gain from migration. A study being undertaken at Colorado State University (DC-48) is concerned with the population changes that have occurred during the period 1950-1970--about half of the counties have been losing population--in the Mountain Region stretching from Montana to Arizona and is considering the consequences of those changes to public finances, retail activity, housing utilization, and agricultural activity. The University of Kentucky (DC-49) is conducting an economic analysis of migration from rural Eastern Kentucky to selected urban centers in Kentucky and Ohio to measure the impact of migration in terms of the provision of selected public services by individual donor and recipient local governments and the Federal Government.

In addition, a field survey of four communities in the Philippines also deals with the relation between migration and fertility but seeks to assess the impact of different types of communities of destination on fertility. This study is being done under contract with the University of Colorado (DC-50).

Research Oriented Toward the Possible Development of Population Policies

A Carolina Population Center (DC-51) study is assessing the knowledge and attitudes of three groups (legislators, key leadership groups, and constituent groups) toward population policies in the area of fertility and population growth and expects to generate suggested programs for developing population policies. In a more specific area, a University of Illinois project (DC-52) is exploring the effect of income on fertility in two different studies: (1) by asking hypothetical questions in a sample survey administered to a regional sample of the U.S. population concerning the effect on personal fertility of government payments for more or frewer children; and (2) by studying the relationship between income and fertility using the 1-in-a 1,000 tape from the 1960 census and demand theory.

Toward the Future

The program of research is being expanded. A number of studies, already approved but not funded due to a shortage of funds, will be initiated in fiscal 1972, and additional studies will be developed as increased funds are available. While such studies as described here will continue to be emphasized, others will be undertaken, for example, to study the effect of population growth and change upon the economy and the environment in an attempt to discover how population growth affects the quality of life.

Each of the studies alluded to above in the general summary is described in greater detail in the summaries of individual projects that follow.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Methodological Study of Measures of Unwanted Childbearing
Contractor: National Center for Health Statistics (reimbursable agreement)
Money Allocated: $11,486 (FY 1970)

Objectives: This study, being conducted by the Institute for Social Research, Temple University, examines ways of formulating questions and the ability of various research techniques to elicit admissions of unwanted childbearing. It investigates interviewing by mail questionnaire, conversational question wording, partial self-enumeration, and traditional personal interview characterized by parsimonious questioning and interviewer enumeration. The study's sample consisted of Philadelphia women who had a second or fifth child during the period January 1967 to June 1968.

Significance to Biomedical Research and Program of the Institute: Given the dependency of much of social research (including that of NICHD's Center for Population Research) on surveys, the construction and administration of surveys is of vital consequence. Moreover, the incidence and prevalence of unwanted childbearing is of considerable importance to the Institute's Population Research Program. This study should aid in the development of questions that can be used in interview and mail surveys that will be conducted for the Center to measure the extent and degree of uncontrolled fertility in the United States. It should also yield considerable practical experience on the conduct of surveys on the family planning practices and attitudes of women who have high fertility and low educational attainment.

Proposed Course: This one-year reimbursable agreement was extended from August 30, 1970 to November 30, 1970 without additional funding to permit the development of additional tabulations requested by the government. A progress report has been received, and the final report is expected.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Fertility and Family Planning in West Malaysia
Contractor: The University of Michigan
Money Allocated: $41,970 (FY 1969)

Objectives: The purpose is to analyze the national probability sample of West Malaysia carried out in 1966-67 by the Malaysian Department of Statistics to determine: (1) patterns of child spacing, (2) age at marriage, and (3) diffusion of contraceptive information.

Major Findings: (1) West Malaysian women have had large families--the average woman 35-44 years of age bore 5.8 children of whom five survived; (2) moreover, starting about 1967 the large groups of females born after World War II began to swell the reproductive age groups and in the absence of very extensive declines in age-specific fertility rates, the crude birth rate will rise; (3) yet a significant decline in fertility was underway even before the start of the national family planning program--two-thirds of the decline in the crude birth rate between 1957 and 1962-1966 was due to real declines in the age-specific fertility rates for married women; (4) survey results point to a likely further decline in fertility and to a successful family planning program; (5) early marriage leads to higher cumulative fertility and multiple marriages, to lower cumulative fertility; (6) rural Indian or Pakistani women have the highest cumulative fertility and urban Chinese women with more than five years of schooling, the lowest; and (7) as to the relationship between education and fertility, 15 or 20 years after marriage the most educated women had significantly fewer children than uneducated women had.

Significance to Biomedical Research and Program of the Institute: It is important to discover the ways in which age at marriage is affected by various influences at work in the society to determine what effect these variations have upon the birth rate and the ways in which the adoption of family planning techniques spreads throughout a society.

Proposed Course: This contract was completed by the end of FY 1970; the final report was submitted in FY 1971.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Variations in Cognitive Styles

Contractor: University of North Carolina
Money Allocated: $32,720 (FY 1970)

Objectives: The purpose is to tape 30 sex information sessions sponsored by Planned Parenthood in Baltimore and to select subjects to be interviewed in order to discover the differences in what instructors think they are saying and what the listeners interpret them to be saying. The tapes and the interviews will be analyzed to abstract dimensions of cognitive style and to establish the congruence and noncongruence of presenter and receiver cognitions. The project aims to describe the types of questions adolescents have, the language they use to communicate about sex, and the types of responses the presenters make to these questions, and to develop an analytic scheme for depicting variations in cognitive style between presenters and receivers and to relate these variations to communication effectiveness. In addition, it seeks to ascertain the sexual behavior of adolescent black youths in a low income urban setting. Its ultimate purpose is to establish guidelines for more effective presentation of sex information.

Significance to Biomedical Research and Program of the Institute: The results of this study will be applicable to sex education programs in this country and abroad and should contribute significantly to the improvement of communication effectiveness in all areas having to do with sex and family planning.

Proposed Course: This contract, begun in 1969, was renewed in fiscal 1970, and will be terminated as soon as a final report is received.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: National Abortion Survey

Contractor: Population Council
Money Allocated: $83,549 (FY 1970)

Objectives: The purpose is to obtain basic information on demographic, socioeconomic and cultural characteristics, experience with induced abortion and attitudes on abortion, by means of a questionnaire administered to a national probability sample of approximately 2,400 women, married and unmarried, 20 through 49 years of age. Specifically, the study is to (a) provide an estimate of the annual number of induced abortions in the United States; (b) evaluate the patterns of induced abortions by age, marital status, number of prior live births and selected socioeconomic and cultural characteristics of the women; and (c) evaluate the pattern of induced abortions by agent and subsequent medical care and hospitalization. It will further assess the perception women have of the incidence of induced abortion among their friends and among women in general, and reassess women's attitudes as to the legalization of abortion under specified circumstances.

Significance to Biomedical Research and Program of the Institute: The incidence of abortion is estimated to be fairly high in the United States, but there are no reliable statistics. Thus, while information of this subject is of great importance, it has been very difficult to obtain. This study, proposed by the most reputable authority on abortion in the United States, is expected to improve considerably our knowledge about the frequency of abortion, the circumstances under which it occurs and the attitudes of women toward the phenomenon.

Proposed Course: The data are currently being tabulated under this one-year contract.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: To Support Training and Experience in the Relationship of the Epidemiology of Reproduction Wastage in Early Pregnancy in the U. S.

Contractor: U. S. Public Health Service, Center for Disease Control, Atlanta, Georgia (reimbursable agreement)

Money Allocated: $535,000

Objectives: This program supports Epidemiological Intelligence Service officers assigned to family planning program evaluation research. These officers receive their basic training and experience in the field under the jurisdiction of the Center for Disease Control. To overcome a paucity of information in a highly dynamic situation relating to abortion policies and practices, the staff of the CDC Family Planning Evaluation Activity have started an "abortion surveillance" program, patterned after earlier and successful efforts to collect, evaluate, and distribute information on communicable diseases.

Significance to Biomedical Research and Program of the Institute: Because of the significant number of women currently taking oral contraceptives and participating in family planning programs today, a sound epidemiologic approach must be developed to evaluate the effectiveness of these programs. This is of immediate interest to this Institute and to the scientific community.

Proposed Course: The agreement, initiated July 1, 1966 to run for one year, has continued in FY 1970 and FY 1971 and is expected to continue in FY 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Physicians' Attitudes Toward Abortion

Contractor: Temple University
Money Allocated: $179,073

Objectives: The major purpose of this study is to assess physicians' current perceptions, understandings, and attitudes toward birth control methods which may be considered as possible abortifacients. The doctors' attitudes concerning criminal as well as legal (therapeutic) abortions and relevant general attitudes toward fertility intervention and birth control will also be examined. Some 1500 interviews will be given to a national probability sample of M.D.'s and osteopaths whose practice makes the subject of fertility control a reasonable occurrence. The interview schedule will be developed by video and audio taping a series of "In-group" discussions with physicians from four regions of the United States: the Northeast, South, Midwest, and West. The tapes will be analyzed and questions concerning how best to approach the subject of birth control and abortion will be determined. The questionnaire will include the following areas: definitions of pregnancy and of abortion; perception of peers and of the public mood toward abortion; individual perceptions of patients and their circumstances toward abortion; etiology in the correlates of attitudes toward abortion and specific objections to abortion; and the actual behavior of the physician regarding prescriptions of birth control methods and when appropriate, the performance of abortion.

Significance to Biomedical Research and Program of the Institute: Since there is a trend toward the liberalization of abortion statutes and the physician is becoming increasingly involved, it is crucial to determine how the medical profession views abortion. This study accordingly has a great deal of relevance to the Institute's program.

Proposed Course: This is the first of two years of funding.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Psychosocial Components of Repeated Abortion Seeking Behavior

Contractor: American Institutes for Research
Money Allocated: $15,025 (FY 1970)

Objectives: The purpose is to investigate the feasibility of studying certain aspects of abortion in collaboration with the Demographic Research Institute of the Hungarian Academy of Science. The study will center on the psychosocial characteristics of women seeking three or more abortions over a period of time, and of a control group of women who have had only one abortion. Special emphasis will be placed on socio-economic factors, education, housing, religion, etc., which may be particularly relevant to the United States. Factors related to the relative use of contraception and abortion will also be investigated. Consultations concerning the feasibility of the joint undertaking will consider prospects of obtaining subjects, availability and suitability of relevant indices and records, opportunities for developing and administering interviews, and psychological assessment devices, staff requirement, cost sharing, and other matters.

Significance to Biomedical Research and Program of the Institute: This study is laying the groundwork for the study of contraceptive practice and abortion seeking behavior in the United States and has important implications for health and contraceptive practice in this country as abortion laws become more liberalized.

Proposed Course: The study, initiated in FY 1970, continued in FY 1971, and a short extension to August 1971 has been requested.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Illegitimacy - Demographic and Sociological Studies

Contractor: University of California
Money Allocated: $87,483

Objectives: The purpose is to develop new information about illegitimate births and expand the methodology available for further study of illegitimacy and related phenomenon. The study, dependent in large part upon available vital and health records, will include the evaluation of California State data on illegitimate births and an analysis of the incidence data for the years 1966 and 1967. The study also includes record linkage cohort studies and an evaluation of interview studies relating to the phenomenon. On the basis of the data to be accumulated, the study will: 1) evaluate State data on illegitimate births; 2) estimate the incidence and distribution of illegitimate births; 3) study the antecedents and consequences of out-of-wedlock pregnancies, as shown by linked vital records; and 4) investigate the feasibility of surveys of attitudes and behaviors in relation to non-marital unions and illegitimate births.

Significance to Biomedical Research and Program of the Institute: This study represents a much-needed basic investigation concerning information which is difficult to get but bears upon a persistent social problem with great significance to the health of both women and children. It will also contribute to the methodology of studying these phenomena.

Proposed Course: The contract initiated in FY 1970, has been renewed with funding for FY 1971.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: New Estimation Techniques for Demographic Analysis

Contractor: University of North Carolina
Money Allocated: $75,755

Objectives: The purpose is to develop sensitive indicators of change in natality patterns. Its specific objectives are to develop and assess appropriate measures for the analysis of "closed" intervals between births and "open" intervals since the most recent birth. Such data are assuming increasing prominence as predictors of natality change and indicators of the effects of family planning programs. The contract will develop new estimators, assess their accuracy, reliability, and sensitivity, and compare various procedures for collecting appropriate data. To evaluate the estimators, an existing microsimulation model will be modified and other special purpose models utilized as necessary. The project should contribute also to more general methods of analyzing current natality data and to the choice of appropriate indices of change.

Under this contract, experiments using augmented simulation models will be devised to (a) simulate reproductive performance, postulating different behavior patterns reflecting different levels and rates of population change, (b) simulate different schemes of sampling in data collection in real populations, (c) calculate from such simulated data the values of the estimators derived above, and (d) assess the behavior of these estimators in response to the conditions of the experiments.

Significance to Biomedical Research and Program of the Institute: The development of more sensitive indicators of current changes in fertility behavior has important implications for both the collection of vital statistics and the evaluation of the effectiveness of Public Health programs, including those relating to family planning.

Proposed Course: The contract, initiated in FY 1970, has been renewed with funding for FY 1971 ($82,817) and FY 1972 ($90,059).

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Preliminary Study of Organization and Procedures Required for Social Science Use of Extended Census Data

Contractor: University of Pennsylvania
Money Allocated: $24,180

Objectives: This proposal will specify procedures for reducing the cost and complexity of dealing with tapes produced by the Bureau of the Census in connection with the 1970 census. The cost of a complete data set as issued on tapes by the Bureau of the Census of approximately $100,000, makes this data base largely inaccesible to the great mass of research workers in academic government, and consulting institutions. This study covers the planning stage of a much larger project to process and distribute 1970 Census Summary Tapes among academic, government and business users who would probably not otherwise have the resources or computer staff and capability to manage the 1970 Census Tape for purposes of national cross tabulations or comparisons. This project will produce detailed specifications of: 1) basic methods to be used to reduce the volume and costs of census data tapes; 2) basic methods to be made available for census tape processing in independent institutions; 3) a review of organizational measures and their feasibility, including (a) the formation of consortiums for the production and distribution of usable data tapes and processing methods, (b) feasibility of cost reduction through private support, (c) feasibility and desirability of foundation support, including that of government foundation type agencies,&(d) recommended organizational forms for a continuing large scale project.

Significance to Biomedical Research and Program of the Institute: Making summary tapes of the 1970 census available is of singular importance to the Institute's program in relation to the population research community.

Proposed Course: This contract is funded for one year into fiscal 1972, with future years to be negotiated.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Social and Economic Variation in Marriage and Fertility Histories, 1967
Contractor: U. S. Bureau of the Census (reimbursable agreement)
Money Allocated: $23,000 (FY 1970)

Objectives: The purpose is to make available to the research community detailed tabulations of new and unique data from a large U. S. sample on the relationship between economic status, nuptiality, family breakdown and fertility. Relevant data collected in the Survey of Economic Opportunity of 1967 have never been analyzed.

Major Findings: (1) first births per 1,000 women in the ten-year period following marriage rose from 692 for white women marrying in the economic depression period 1930-1939 to 905 for those marrying in the period 1955-1959--and births came sooner after marriage for the latter cohort; (2) premarital first-birth rates also rose in this period; (3) age at first marriage is inversely related to lifetime fertility; (4) poor wives were younger at first marriage, had children sooner after marriage, and had larger families than other wives--their expectations of fewer future births accordingly may reflect a less realistic outlook; (5) wives who are not high school graduates expect more children than do wives with more education; (6) urban-rural fertility differentials have lessened, and birth expectations data for wives 30-39 years of age indicate a further narrowing in terms of the average number of children ever born; and (7) the data suggest that declines in the average size of completed families will occur in the near future and will continue, e.g., three-fourths of white wives 14-39 years of age who have already had two children expect no more children.

Significance to Biomedical Research and Program of the Institute: This project is highly relevant to the Institute's goal of better understanding the determinants of fertility in our society and particularly of the relationship between marital status and fertility in a time of rapid change.

Proposed Course: Findings were reported in "Previous and Prospective Fertility: 1967," Population Characteristics, Series P-20, No. 211, January 26, 1971, U. S. Bureau of the Census.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Investigation of the Determinants of Fertility in the United States

Contractor: The Rand Corporation, Santa Monica, California
Money Allocated: $118,300

Objectives: This research will attempt to identify the principal social determinants of family size and current fertility patterns in the United States. A set of interrelated hypotheses are offered to account for systematic components of the variation in parent and group reproductive behavior and to explore the relationships between fertility and several related types of behavior. This model will be tested and refined by linear and non-linear regression analysis using several sets of individual family data for the United States; (1) Survey of Economic Opportunity, 22,000 households, 1966-1967; (2) Longitudinal Survey of Family Economics, 3,000 families, 1967-1970; and (3) Time Budget Survey, 2,000 families, 1964.

Given the biological probabilities of bearing a child a function of age and previous childbearing experience, the study will determine the effects of various factors such as income, time of marriage, female labor force participation and timing and frequency of migration and characteristics related to these phenomena. It will try to describe with increasing precision the behavioral and biological mechanisms affecting fertility and ascertain how they differ among various segments of the U.S. population. Within a relatively comprehensive framework of analysis the influence of socioeconomic factors on fertility should provide the basis for estimating the probable impact on the birth rate of: (1) increased years of schooling; (2) unearned income supplements to the working poor; (3) change in female to male relative earnings (after taxes); (4) change in tax deductions for dependents; and (5) housing and rent subsidies.

Significance to Biomedical Research and Program of the Institute: The study of determinants of fertility and the development of related methodologies are highly relevant to the Institute's work. This offers the possibility of increasing our knowledge of the determinants of fertility and their inter-relationships through the statistical analysis of several existing bodies of data.

Proposed Course: Total funding for the eighteen-month project is $180,000.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Analysis of the Decline of Fertility in Europe, by Province

Contractor: Office of Population Research, Princeton University
Money Allocated: $101,160

Objectives: The purpose is to analyze the decline in fertility in each of the more than 700 provinces in Europe from the time that marital fertility was essentially constant until the present, or until it reached a minimum and began to rise. Its aim is to determine the circumstances, in terms of the education, religion, occupational structure, residence patterns, etc., of the population, under which fertility declined. This study will make an essential contribution to the social history of Europe and should elaborate considerably upon our understanding of the factors relating to the decline of fertility.

Significance to Biomedical Research and Program of the Institute: Fertility has been designated as an area of the highest priority by the Center for Population Research. This study will furnish information in far greater detail than has previously been available on the social and the economic factors associated with the decline in fertility in many countries of Europe over the past two centuries or so.

Proposed Course: This project, underway for several years, was extended into fiscal 1971.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Processes of Demographic Development in Imperial Russia and the Soviet Union

Contractor: The Ohio State University
Money Allocated: $52,357

Objectives: The purpose is to study the mutual relationships between population growth, economic and social change in Imperial Russia and the Soviet Union. In order to identify the long-term factors that determine and influence demographic changes, the study will explore the determinants of Soviet population growth from a long-run point of view, which also includes the early stages of change during the Imperial Russian period, and to appraise the Soviet efforts to design and implement population policies to influence fertility patterns and other demographic variables. The principal themes of the proposed research will be those concerned with the implications of the developmental process for population growth, with major attention given to the question of fertility.

Significance to Biomedical Research and Program of the Institute: The study of demographic developments during a process of economic development and social change during the 18th, 19th, and 20th centuries in a society which shows both European and Asiatic influences is of the highest relevance in understanding the process of population change in the world at large. The findings will increase knowledge of the factors associated with declining fertility, the use of contraceptives and of abortion, and will provide some insight into the effectiveness of a variety of population policies.

Proposed Course: The original contract, initiated in 1970, has been renewed with funding for FY 1971 ($52,357) and FY 1972 ($54,798).

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Human Fertility in Poland and the U.S.S.R.

Contractor: Western Washington State College
Money Allocated: $22,979

Objectives: The primary objective is an investigation of social, political, and economic influences bearing on fertility in Poland and the U.S.S.R. and including rural fertility differentials by size of land holding, and abortion and fertility differentials by employment status of women in Poland, and the social and economic correlates of fertility and birth control in the U.S.S.R.

Since the mid 1950's the Soviet Union and the Eastern European countries within its sphere have published more demographic and economic data and have liberalized abortion laws (partly because of limited housing and the need to increase the number and proportion of females in the labor force).

The demographic processes in the Communist countries need to be studied for the light they may shed upon relationships of population to socio-economic-political conditions which are both similar to and different from those in other regions. Fertility differentials between the more developed parts of the RSFSR on the one hand and the five Union Republics of Central Asia and of the Caucasus, and of the autonomous republics on the other hand should throw additional light on basic demographic processes in various cultural contexts. In addition, the effects of sharp and contradictory policy changes need exploring both to assess the effects of such shifts and to avoid similar problems in the U.S.

Significance to Biomedical Research and Program of the Institute: All aspects of the research are program relevant, but particularly the impact of abortion, of urban-rural, religious and other ethnic differentials and, more generally, of "modernization," on fertility trends. Moreover, the investigator is one of a very small number of persons in the U.S. with both the language and technical skills to pursue studies in the Slavic countries.

Proposed Course: This is the first of two years of funding.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Socio-economic Factors in the Reduction of Natality in the Less Developed Areas

Contractor: Food Research Institute
Stanford University
Money Allocated: $68,539

Objectives: The objective is to examine the usefulness of the notion of the demographic transition, or modifications thereof, as a model for fertility reduction in non-Western areas, and to determine the context of socio-economic conditions that appear to be the basis for the onset of a decline in fertility in the underdeveloped or developing countries of the world. Measures of natality and of socio-economic development are being studied in each of the several major regions of the world to determine their accuracy and comparability, and to analyze their interdependence. The study will also seek to provide a possible measuring stick or projective device for estimating the effects of efforts to reduce fertility in the future.

Significance to Biomedical Research and Program of the Institute: This study will provide insight into the process of fertility decline in the developing and underdeveloped regions of the world and the extent to which the socio-economic conditions associated with natality decline in the West are having, or are likely to have, the same effect in the non-Western world, as well as indicating what additional factors may influence fertility decline in these areas.

Proposed Course: This contract, begun in FY 1970, has been renewed with funding for FY 1971.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Urbanization, Migration and Fertility in Thailand
Contractor: Brown University
Money Allocated: $45,887

Objectives: The purpose is to analyze census data and data from a national longitudinal survey of Thailand to determine the relationship between urbanization, migration, and fertility. The fertility data available from this national survey, cross-tabulated by residence and household type, permit their evaluation in terms of a rural-urban continuum, and their control for age differentials provide the opportunity for comparisons of fertility levels across the urban-rural continuum for differentials by religion, literacy, education, economic status, migration, and marital status.

More specifically, the study will analyze: (1) cross-sectional and trend data on urbanization and on the relationship between fertility and socio-economic variables based on extensive data collected in the 1960 Thai Census and preliminary data from the 1970 Census; (2) rural and urban data from the National Longitudinal Survey of Social, Economic, and Demographic Change in Thailand collected 1968-1970 to ascertain: (a) total migration experience of the population: (b) the role of population and economic pressures on movement away from the village of birth; and (c) the relation between migration and fertility; (3) rural-urban labor force, occupation, and industrial differentials and data on literacy, education, and religion; and (4) data for integrating the material from the rural and urban survey operations.

Significance to Biomedical Research and Program of the Institute: The study will add to available knowledge of rural, urban, and other socio-economic differentials in fertility in an area for which data have previously been nonexistent, and for a culture which is quite different from that in the United States. Hence, it will add another perspective to the understanding of influences upon fertility behavior.

Proposed Course: The contract, initiated in 1970, is anticipated to run for a period of three years.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Economic Cost and Value of Children in Four Societies

Contractor: Columbia University

Money Allocated: $73,050

Objectives: This research is directly aimed at the assessment of the importance of economic costs of, and returns from, children as possible factors in fertility decisions. The secondary aim will be the development of refinement of research methodology for such studies.

Peasant communities in Indonesia, India and Mexico and a farming community in the United States will be studied using anthropological techniques of intensive observation together with extensive questionnaire surveys. Field investigation and data analysis will be carried out by four research assistants familiar with the particular communities in which they will be doing the research. Intensive observation of domestic and productive activities will be used to assess the costs and benefits associated with different family sizes in each community. Census and fertility surveys of the four populations will be carried out to determine actual fertility and attitudes regarding fertility. Data analysis in the third year will provide comparable data for all societies.

An accurate census will be taken with respect to demographic information, socio-economic status, land holdings, etc. The communities studied will each comprise about 400 households of which a sample will be selected for intense study to include: (a) households in different stages in the developmental cycle, etc.; and (b) the organization and productivity of labor, and the economic cost of children, both direct and indirect. A third sample will be comprised of all married women of reproductive age or a randomly drawn sample of such women with interviews to be based on the short list of variables for comparative fertility studies from the International Union for the Scientific Study of Population.

Significance to Biomedical Research and Program of the Institute: This study deals with a crucial area of population research that has definite relevance to the Institute's program. Very little information has been available on the economic cost or benefit of children and how these are perceived, even for industrial societies.

Proposed Course: This is the first of three years' funding. Approved funding for FY 1972 is $81,590 and for FY 1973 is $87,056, making a three-year total of $241,696.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: National Fertility Survey, 1970-1975

Contractor: Princeton University
Money Allocated: $519,340

Objectives: The objective is to survey approximately 6,500 women representing a national sample of all ever-married women under age 45 in the coterminous U.S. to determine fertility behavior and fertility expectations of American women. The study is a continuation of studies begun as the Growth of American Families Study, 1955, repeated in 1960, and of a similar National Fertility Survey done by the present investigators in 1965. The study will compare fertility behaviors and expectations by age, marital duration, race, education, income and religion, and will investigate the use of various contraceptive methods, and the influence of a broad range of factors on their use. It will include for the first time a sample of women previously married but not currently in the married, husband-present category. Data developed in this study will be augmented to overcome shortcomings observed in the previous studies, e.g., by analyzing fertility by duration of marriage as well as by age.

Significance to Biomedical Research and Program of the Institute: This study will continue a unique series of studies on fertility, fertility expectations and contraceptive practices among women in the U.S., and will provide a base of reference for prospective studies on contraceptive practices and the influences upon the health of women and children. It is an essential part of the public health aspect of the study of contraceptives as well as of attitudes toward family size.

Proposed Course: This contract, initiated in 1970, was continued in fiscal 1971.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Sex Role, Family Structure, and Fertility Control

Contractor: Indiana University
Money Allocated: $27,681

Objectives: The purpose is to study the relationships between definitions and perceptions of male and female roles within the family and achieved and anticipated fertility. The specific concern of the study is with those decisions relating to childbearing and with the antecedent conditions that structure them, with emphasis upon the husband-wife interaction and, particularly, with reference to the alternative sets of roles and rewards which are perceived as valued and open to the female. Emphasis will be placed upon the effect of wife's employment and career orientation upon decisions with respect to the size of family. The contract involves the interviewing of a regional probability sample of 3,000 persons, 15 to 45 years of age, drawn from the East North Central Region of the United States.

Significance to Biomedical Research and Program of the Institute: This contract bears on many aspects of the Institute's concern with contraceptive practice and family planning: age at marriage, decisions affecting fertility, alternatives to childbearing, such as employment of the woman, probability of divorce, and socialization for marriage and parenthood.

Proposed Course: This contract, initiated in FY 1970, has been renewed with funding for FY 1971 ($27,681) and FY 1972 ($10,244).

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Self Identity of College Women
Contractor: Institute for Juvenile Research, Chicago
Money Allocated: $40,998

Objectives: The purpose is to study changes during the freshman year in college in the sexual identity of girls, particularly appropriate subjects as they are much concerned with balancing work involvement and social-sexual involvement.[1/] Before entering college all incoming freshman women will be given a questionnaire designed to measure self-concept, sexual identity values and life goals. During the first year, periodic interviews and individual and personal logs will be used to trace changes in the previously measured characteristics. The role of the value climate of the college, reference figures and groups in the socialization of female sexuality will be studied.

Significance to Biomedical Research and Program of the Institute: This study assumes that the way in which sexuality is integrated into the girl's structure of identity and motivation determine immediate and longer-run decisions regarding work and fertility. Thus this research is highly relevant to the Institute's program, with its emphasis on greater understanding of the interrelationships among female sexuality, fertility, and career and life goals.

Proposed Course: It is expected that the work on this contract will be completed during fiscal 1972.

1/ Sexual identity is defined as having certain aspects including (1) gender identity, (2) body image, (3) "masculinity-femininity," (4) feelings of attractiveness and popularity, (5) sexual initiation and intimacy, and (6) fertility and maternity.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Women's Labor Force Participation and Fertility

Contractor: Research Triangle Institute

Money Allocated: $110,664

Objectives: This two-phase study is based on a complex causal model of the relationship between women's fertility and their labor force activity. The model suggests that antecedent social and economic variables determine labor force participation and fertility and that labor force participation affects fertility only indirectly. It will not duplicate work underway at the University of Wisconsin.

Phase I entails the secondary analysis of at least two of four previously collected data sets which report on the fertility behavior of American women of childbearing ages: (1) Growth of American Families Survey, 1960; (2) Survey of Work Experience, 1967-70; (3) Family Growth in Metropolitan America Survey, 1957-60; and (4) the Survey of Economic Opportunity, 1966-67. Data from the Survey of Work Experience and Survey of Economic Opportunity will be used to test the hypotheses resulting from the causal model and allow the researcher to formulate a design and instrumentation for the second part of the study. Phase II will develop and administer an interview schedule to a sample of 300 women and a sub-sample of 150 of their husbands. The pilot study will attempt to develop new techniques for determining attitudes relevant to fertility behavior not covered sufficiently in the above studies and will serve as a check for the causal model developed. If these two phases of the study are completed satisfactorily, the principal investigator plans to submit a proposal entailing a national sample.

Significance to Biomedical Research and Program of the Institute: The proposal deals with a very crucial area of population well within the Institute's interests. There has been much written about the relationship between women's labor force participation and its influence on fertility behavior, but there is still much confusion about this relationship. This proposal approaches the problem in a sophisticated, fresh manner, and even if the causal model does not work, the descriptive data amassed will allow researchers to construct new conceptual and methodological approaches.

Proposed Course: This is the amount requested for 14 months, but upon Panel recommendation the project is being stretched out to a total of two years.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Correlates of Low Fertility in a Developing Country

Contractor: Wayne State University
Money Allocated: $29,677

Objectives: This study will examine the antecedent factors to explain how the level of urban Greek fertility can be low in the absence of (a) organized birth control and other governmental programs, and (b) economic industrial development.

Data already collected in the Greater Athens Metropolitan Area in 1966-67 will be analyzed to study: the relationship of the employment of women to fertility and birth control, examining three aspects of female employment: (a) the degree of compatibility between the working and mother roles, (b) the type of occupation, and (c) the degree of work commitment.

Significance to Biomedical Research and Program of the Institute: The central problem of trying to account for the low birth rate in Greece without much industrialization or family planning programs is of considerable theoretical and possibly practical significance. The project is highly relevant to the Institute's interests in the determinants of fertility.

Proposed Course: It is expected that the work on this contract will be completed during fiscal 1972, and that there will be no need for renewal or extension of the contract.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Differentials and Trends in Marital Disruption, Remarriage, and the Fertility of Remarriage

Contractor: University of Wisconsin
Money Allocated: $42,378

Objectives: The purpose is to examine patterns of marital disruption, remarriage, and the fertility of remarriages in the United States from data obtained in the 1967 Survey of Economic Opportunity, which involved a large sample and covered the national population. The study will be concerned with (a) differentials among socio-economic groups and racial groups, (b) the patterns of divorce and widowhood in relation to age, marriage, duration, color. education, age at marriage, order of marriage, sex, and presence of children, as well as place of residence, and the length of the interval between marriage and the birth of the first child, and with patterns of remarriage in relation to the above; and (c) how these patterns have changed through time. It will also investigate the pattern of fertility of second and subsequent marriages and how differential fertility and change in fertility is influenced by differential marital disruption.

Significance to Biomedical Research and Program of the Institute: This study contributes to the understanding of the role played by marital disruption and remarriage in the practice of contraception and attitudes toward family size.

Proposed Course: This contract, initiated in FY 1970, has been renewed with funding through FY 1971.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Social-Psychological Correlates of Urban Fertility
Contractor: Bowling Green State University
Money Allocated: $70,830

Objectives: This research, involving a re-interview of 754 mothers interviewed in Toledo in 1963 and a sample of 3,000 married women in the Toleda SMSA, has several interdependent objectives designed to test for possible associations, within broad religious and socioeconomic categories, between selected social-psychological and demographic variables: (1) to determine by means of longitudinal study the degree to which present family size can be predicted from alienation scores obtained from the sample of mothers in 1963; (2) to determine the extent to which the obtained differentials in fertility by alienation variables in previous research would be accentuated and refined in a community-wide sample that would include mothers who are continuing their childbearing as well as mothers who have successfully terminated their fertility; (3) to identify some of the social and demographic factors associated with differential kinds and degrees of alienation among mothers at given age, parity, and marital duration levels; (4) to determine the extent to which selected husband-wife interaction patterns are associated with differential alienation-fertility relationships; and (5) to derive a conceptual model of differential family planning and fertility behavior by combining stratification, religion, mobility, alienation, family, and information variables.

It will concentrate on attempts to understand better fertility and family planning differentials within social class and religious categories. Alienation variables will be considered as intervening between social structures and fertility, and the primary emphasis will be on specifying the conditions--of education, religion, age, etc.--under which particular patterns of alienation dimensions may be causally related to the dependent variables.

Significance to Biomedical Research and Program of the Institute: A study of the social psychological factors affecting fertility will make an important contribution to our understanding of fertility behavior, the sine qua non of the Institute's program in population research.

Proposed Course: Funding for this contract is for one year; future years would be negotiated.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Relationship between Age at Marriage, Birth Intervals and Total Fertility

Contractor: U.S. Bureau of the Census (reimbursable agreement)
Money Allocated: $225,000

Objectives: This project will permit the collection, tabulation, research and analysis of data on fertility of American women, with specific emphasis on data reflecting marriage, fertility and childspacing relationships. Designed to increase our knowledge of timing patterns of births and of factors that explain or bring about changes in timing, it includes research and analysis of current fertility levels and trends, the ways of using specific types of data for measuring changes in timing patterns of childbearing, ways of applying such knowledge to fertility projections, and exploration of new kinds of data needed to increase our understanding of recent fertility trends. A series of questions will be added to the Census Bureau's Current Population Survey questionnaire to obtain information on year of marriage, number of children ever born, birth order, and time interval between birth orders, to provide the basic data needed to develop childspacing patterns and to determine how they have been changing. The 50,000 household sample of the CPS will provide Nationwide statistics on current timing patterns as compared with corresponding data collected in former years (1960 and 1965).

Significance to Biomedical Research and Program of the Institute: This study will make an important contribution to the analysis of trends in fertility, by analyzing age at marriage and timing of births (childspacing) within marriage in relation to levels of fertility. An immediate objective is the development of a methodology applicable to fertility projection, but more broadly, it will also provide basic information for an understanding of fertility behavior and its changes in the United States over time. The project thus bears directly upon a primary concern of the Institute's program.

Proposed Course: Funding is for one year, with future years to be negotiated.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Mating Patterns in Seven Latin American Capital Cities

Contractor: University of Massachusetts
Money Allocated: $13,750

Objectives: This study provides for the final tabulation, analysis, and interpretation of data from a fertility survey carried out in seven Latin American capital cities in 1964 under the guidance of the United Nations Demographic Center located in Santiago, Chile (CELADE). The survey produced data valuable for the investigation of: (a) female ages on first entering sexual union and (b) interruptions of sexual unions, both of these being immediate determinants of fertility. The research treats both age on entering unions and interruptions of unions as dependent variables. It seeks not only to determine their present levels and recent trends, but also to understand their determinants in order to better predict future trends.

Significance to Biomedical Research and Program of the Institute: This subject is clearly relevant to the Institute's program, since age at first entry into sexual union and interruptions in unions due to separations, widowhood, etc., are important factors affecting fertility.

Proposed Course: This is the first of two years' funding; future years are to be negotiated.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Correlates of Family Size and Expected Family Size Among Puerto Rican Youth

Contractor: Boston College
Money Allocated: $47,189 (FY 1970)

Objectives: The purpose is to examine the differential characteristics of large and small families in Puerto Rico. The study seeks to determine how parents who have small families differ from those having large families in such characteristics as socio-economic status, urban origins and residence, vertical and horizontal mobility, maternal employment, participation in voluntary organizations, and relationships with relatives. It also seeks to discover the differences between children from small and large families in terms of such characteristics as perception of parents and parent-child relationships, academic achievement, family planning, age at marriage, educational planning, social participation, and other personality factors. And, finally, it seeks to discover how children and young people who intend to plan their families differ from those who do not intend to do so, in terms of such factors as socio-economic class, religion, degree of religiosity, school attended, size of family of origin, occupational plans, planned age at marriage, and selected personality factors.

Significance to Biomedical Research and Program of the Institute: The study will extend knowledge of the psychological, social and economic factors related to attitudes toward family size and the practice of contraception and thus contribute importantly to the Public Health aspect of the study of contraceptives.

Proposed Course: This study, initiated in FY 1970, will be completed as soon as the final report is received.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Causes and Consequences of Sex-Age Differences in Human Populations

Contractor: University of California, Berkeley
Money Allocated: $42,488 (FY 1969)

Objectives: The purpose of this study is to collect data from as many countries as possible on the following: 1) sex ratio at birth, 2) sex ratios at successive ages, 3) sex differences in mortality, and 4) marriages by age of groom, to determine first of all, the statistical uniformities among these data and to explain them in social and cultural terms. A further objective is to experiment with models which will display the relationships between the various data when changes occur in one set of data (for example, the sex ratio) to see the extent to which they affect relationships among the other variables.

Significance to Biomedical Research and Program of the Institute: This study will have important policy implications and open new areas for further demographic analysis with respect to problems relating to natality differentials, disproportion of widows and widowers, varying marriage patterns and marriage markets, labor force composition, migration and other demographic matters.

Proposed Course: Work under this contract, begun in 1969, was extended without additional funds and will be terminated as soon as the final report is received.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Attitudinal Factors Affecting Family Size

Contractor: Worcester Foundation for Experimental Biology
Money Allocated: $44,085

Objectives: This research (1) tests the hypothesis that women who perceive little difference between sex roles and who reject (for themselves) the stereotype of women as less assertive, ambitious, intelligent, etc., than men are the ones most likely to accept employment and to desire and achieve relatively small family size; and (2) the "perceived sex-role differences and self-concepts" will be related to "antecedent biographical characteristics and family backgrounds". The second goal of the study will provide information for a later longitudinal study. The sample will include 1,000 persons--375 married men, their 375 wives, 125 single men and 125 single women of evenly distributed ages (17 to 55) and socioeconomic levels (based on husband's occupation or, for younger men, education). The subjects will be obtained from local civic, professional, religious, and labor union groups and will be selected to be representative of the community.

Members will fill out the questionnaires in group sessions. One questionnaire calls for rating 122 tested items three different ways, to diagnose male and female "sex-roles" as perceived for men, for women, and for themselves. The other questionnaire covers background data, employment and occupational history--including attitudes toward employment, achieved and desired family size, and contraceptive attitudes and practices. There are also questions about plans for working at the time of marriage, the factors that determined entry into the labor force or withdrawal from it, ideal family size and family planning orientation.

Significance to Biomedical Research and Program of the Institute: This study of several aspects of the relationship between achieved or planned fertility and the perception of the sex role of women has importance to the understanding of fertility behavior, which is crucial to the Institute's mission.

Proposed Course: This contract is funded for two years, with future years to be negotiated.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Investigation of the Factors Involved in the Moral Judgment of Marital Status and Family Size
Contractor: Human Resources Research Organization, Fort Knox, Kentucky.
Money Allocated: $32,118

Objectives: Some 500 middle class persons (100 of 16-17 year olds, 100 persons 18-20 years of age, and 300 adults divided among younger and older groups) drawn from ten organizations (PTA's, unions, alumni associations, Catholic groups, etc.) will be asked to respond to situation descriptions presenting marital status (single, married) and family sizes (0,2,6 children) under varied conditions of choice or choicelessness: personal desire, ability (power), and environmental circumstances. Respondents will describe their family status and how they achieved it. Various characteristics of the judges will be studied for their impact on judgments. Perceived attractiveness of the subjects judged will be examined (by bi-polar scale) to determine effects of moral judgment on this dimension. The study assumes that moral judgments are key factors in social pressures to conform, that single people and childless couples are widely viewed as blameworthy, and that this is deleterious to population control efforts. Analysis of variance will be performed to determine the effects of each of the three factors (from Heider) on moral judgment.

Significance to Biomedical Research and Program of the Institute: An understanding of population dynamics relative to future changes in the U. S. population requires a knowledge of attitudes toward family formation. This involves not only the couple but also the social context in which they live. Historically, parents, relatives, and friends have brought pressures on the couple to marry and to start childbearing early and to have large families. Thus it is highly relevant to the Institute's program to explore moral judgments as a framework for evaluating the effectiveness of programs designed to alter attitudes toward marriage or family size. The findings should be useful in counseling situations, family planning clinics, in wider educational programs dealing with marriage and family size, and in welfare programs. They should also contribute to improved maternal and infant health to the degree that they would foster changed attitudes and action programs designed to encourage the spacing of children at longer intervals.

Proposed Course: It is anticipated that this project will be completed within fiscal 1972 and that there will be no renewal.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Kin Network and Population Dynamics

Contractor: Harvard University
Money Allocated: $14,980

Objectives: This study will collect data on the extent and relevance of kin networks in the United States to determine their consequences on fertility and migration. It is hypothesized that the greater the intensity of the kin network (frequency of contact, etc.) and of the interchange therein, the higher the fertility. The kin network may also influence the amount and direction of migration. A conference of persons experienced in the family and fertility will be convened to approve and amend the trial questionnaire and another to evaluate the results. The pretest will be conducted by the U.S. Bureau of the Census.

Significance to Biomedical Research and Program of the Institute: Research on the relationship of the kin network to fertility has high program relevance. Very little work in this direction has been done.

Proposed Course: First year funding; requested funding for two additional years for administration by the Bureau of the Census of a revised questionnaire to a sample of respondents in the monthly Current Population Survey is subject to negotiation following an evaluation of first year results.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Effects of Purposive-Rationality, Traditionalism, and Economic Aspirations on the Process of Family Formation

Contractor: University of Kansas
Money Allocated: $19,988

Objectives: This research specifies a unique data collection procedure involving hand delivering to the household questionnaires which are then self-administered and returned by mail. It would study 1,000 first-time parents to find factors affecting decision making as to the number and spacing of births. The independent variables are: (1) purposive-rationality, (2) traditionalism, and (3) economic aspirations as orientations affecting fertility (dependent variable). These independent variables should provide a parsimonious system with a high degree of explanatory power (e.g., as contrasted with the status variables of SES, education, and religion).

Significance to Biomedical Research and Program of the Institute: This study of how persons make decisions concerning family formation is definitely relevant to the Institute's program. The "training" of respondents to use a self-administered questionnaire facilitates followup over time and imparts to this study a methodological as well as substantive importance, since one of the problems with longitudinal studies is their very great expense. If this methodology works, it can serve as a model for other longitudinal studies at more reasonable cost.

The project should make contributions to the sociology of the family and be particularly helpful in understanding how and why persons decide to have different numbers of children. As a result, it should prove useful for persons in the area of family planning.

Proposed Course: This is the first of three years' funding with a modest total of less than $45,000. Approved funding for FY 1972 is $18,663 and for FY 1973 is $5,796.

Contract Title: Migration Differentials in the United States

Contractor: University of Massachusetts
Money Allocated: $46,600 (FY 1969)

Objectives: It is the purpose of this study to examine the differentials in internal migration in the United States and to determine the impact of these differentials upon sending and receiving areas. The first stage of the contract consists of preparation of a monograph which summarizes the literature on the differentials in migration, by age, sex, color and race, marital status, fertility, physical and mental health, intelligence, education, occupation and related factors. The second stage will consist of an analysis of mobility data for the years 1955-1960 from the 1960 census of population, focusing primarily on education and color differentials, against the background of differentials as developed in the monograph. In the third stage of the study attention will be focused upon the impact of differentials in migration upon the receiving and sending areas with special emphasis upon the largest cities and upon rural areas. The overall purpose is to contribute to the understanding of recent social change and furnish materials for an extension of migration theory.

Significance to Biomedical Research and Program of the Institute: Since migration can have immense impact upon both receiving and sending areas (i.e., depopulation and diminution of services in rural areas and the concentration of inadequately trained people in cities where facilities are inadequate to care for them) the analysis proposed in this study can be of great benefit to policy makers and program planners having to do with the affected areas.

Proposed Course: This contract is completed except for the final report.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Determinants of Fertility, Migration, and Population Change of Counties in the United States, 1950 to 1960

Contractor: University of Georgia
Money Allocated: $40,781

Objectives: This study will analyze the trends and patterns of population change for the total white and nonwhite populations of all counties in the United States from 1950 to 1960. Changes in this period will be explained in terms of fertility, mortality, and net migration, the major emphasis being on fertility and migration and upon the white and nonwhite differentials. The study also investigates the socio-economic and demographic determinants of each of the three components of population change among both white and nonwhites. County fertility rates and differentials, for example, will be examined by age (& age standardized), sex, color composition, and by levels of urbanization, industrialization, income, occupation, education, and related factors. Finally, tabulation and organization of the data by color, not previously compiled on a uniform basis will provide benchmark data for all counties in the U.S. for the 1950-1960 period for comparison with similar trends in the 1960-1970 decade. Plans have already been made for future computatron of the 1960-1970 components of county population change by color. Maps will be prepared showing county birth, death, and net migration rates, and natural increase rates, by color. Birth rates of counties which gained and lost population will be systematically examined, and constellations of counties with similar fertility and net migration patterns will be identified.

Significance to Biomedical Research and Program of the Institute: This efficient utilization of massive data already processed, and coupled with further data from the censuses and the county and city data books, for the 1950's is a useful approach to the analysis of population change in clusters of counties and regions of the United States, and is basic to a projected analysis of the dynamics of the 1960's. This study is exceedingly relevant to the study of population processes in the United States and, therefore, to the Institute's program, both from a descriptive and an analytical point of view.

Proposed Course: This is the first year of funding for a two-year project.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Relation of Fertility to Migration and Selected Socioeconomic Factors
Contractor: Louisiana State University
Money Allocated: $29,960 (FY 1969)

Objectives: The purpose is to study the relationship between fertility and migration status in data from the 1960 census of population relating to migration which occurred during the period 1955-1960. Emphasis will be placed upon the influence of socioeconomic factors on fertility to the origin and destination of migrants, and the relationship of female employment to fertility and migration.

Significance to Biomedical Research and Program of the Institute: The relationship between fertility and migration is important in determining levels of fertility in specific areas and in determining to what extent high fertility hampers, or low fertility enhances, spatial and social mobility. It has additional theoretical implications in that whatever relationships are ascertained will be useful in determining interrelationships between fertility, social and economic factors and migration.

Proposed Course: This project has been completed except for the submittal of a technical appendix relating the problems of classifying migration through the usage of census data.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Analysis of the Spacing of Pregnancies, Births, and completed Fertility in Relation to Socio-economic Status, Intergenerational Mobility and Aspirations for Children, Aberdeen, Scotland

Contractor: Brown University

Money Allocated: $24,523

Objectives: The data are based on a probability sample of resident women of Aberdeen, Scotland, who had at least one live birth during the period October 1950-September 1955. The pregnancy histories of these women up to 1964 have been copied from hospital records and will be updated to 1970; the other required information has been obtained by means of an interview survey (2,000 cases). Preliminary tabulations of the survey data indicate that a "significant test of intergenerational mobility hypothesis" is possible with the data at hand and that the "data on mobility aspirations for children are particularly exciting and promising".

The study will investigate: (1) how spacing of pregnancy varies with the type of terminations of previous pregnancies, the stage in the family life cycle, age at marriage, age at first pregnancy, and socio-economic status; (2) how the spacing of live births, the number of children ever born, and the length of fertility span vary by age, age at marriage, age at first pregnancy, socio-economic status, social mobility, and mobility aspirations for children; (3) the life conditions under which women are likely to resort to terminations and sterilizations; and (4) how the size of the family of origin, and position on a traditionalism scale are related to social mobility, present socio-economic status, aspiration for children and fertility.

Significance to Biomedical Research and Program of the Institute: The Aberdeen data provide a rich source to know how the outcome of one pregnancy influences subsequent pregnancies, how family origin variables relate to childspacing, and how fertility relates to social mobility. The analysis of pregnancy histories and the relationship between childbearing and social mobility are thus very relevant to the Institute's program of population research.

Proposed Course: This is the first of two years' funding; future years are to be negotiated.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Socioeconomic Status and Family Factors in Residential Mobility

Contractor: U.S. Bureau of the Census (reimbursable agreement)
Money Allocated: $5,800

Objective: This study will combine data from the March Current Population Survey taken by the Bureau of the Census for the years 1966-1970 to study annual migration during that period by single years of age. It is designed to provide new perspectives on the joint influence of various socioeconomic and familial statuses on the probability of making different types of moves. It will also produce statistics allowing more precise estimates to be made of the number of times an individual would move in his lifetime if he moved according to currently existing age-specific rates of residential mobility. The study will also examine the effects of number and ages of children (simultaneously considered) on the probability of moving. In these comparisons, age of family head will always be controlled, and so will occupation in some cases. Other questions to be investigated include the effect of the wife's labor force status (whether or not she has a job) on the family's geographic mobility when age and occupation of husband are held constant. A related question is the overall effect of occupation on geographical mobility for men and women, when age and marital status are held constant. In most cases the effects of the various socioeconomic and family statuses will be examined for the Negro and white populations separately. Comparison of the annual data on moves with data from the 1970 census based on residence in 1965 and 1970 will be possible.

Significance to Biomedical Research and Program of the Institute: This research will produce information on characteristics of migrants not previously assembled, making possible more refined estimates of probabilities of moving each year, by occupation, education, race, and family characteristics. It is a carefully planned project by a competent investigator, at marginal cost. Study of migration differentials is relevant to the Institute's program, especially as these relate to family status and size of family.

Proposed Course: It is expected that the work on this contract will be completed within fiscal 1972 and that there will be no need for extension or renewal of the contract.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Migration, Employment and Earnings in the States, Metropolitan Areas, Non-Metropolitan Areas, 1957-62 and 1962-67

Contractor: U. S. Department of Commerce (reimbursable agreement)

Money Allocated: $57,600

Objectives: Data from the one-percent sample of reports on the Social Security-covered workforce will be tabulated to provide a data bank from which other investigators can study mobility among the States, SMSA's, and nonmetropolitan areas of the United States. These tabulations would measure inmigration, outmigration, and the nonmigrant workforce of each relevant area, cross-classified according to age, race, sex, area of origin (for inmigrants), area of destination (for outmigrants), industry of employment and level of earnings. The tabulations will show the employment and mean wages for those working in each area according to status as inmigrants, outmigrants, new entrants into the workforce, and retirees. From such tapes changes in employment in any one of these areas may be analyzed with reference to previous employment the year before or for a period of years (1) in that area or (2) in any or all of the other areas, and distinguished by the following variables: (a) all employment, (b) industry classification, (c) age, (d) sex, (e) color, and (f) average wage in the current and previous employment.

Significance to Biomedical Research and Program of the Institute: The redistribution of population and related factors associated with population movements are of the highest relevance to the Institutes program. No other data are as good, and the publication of a good set of tabulations from the OASDI one-percent sample will be a milestone in migration research. The tabulations will be particularly useful in determining the relationship between migration flows by age, color, and sex with respect to economic opportunities both between the states and between particular SMSA's, and nonmetropolitan areas. For example, it will be possible to analyze for such a city as Chicago the sources of migration from other parts of the United States by age, sex, color, and some of the economic factors which underlie these movements.

Proposed Course: It is expected that the work on this contract will be completed during fiscal 1972, and that there will be no need for renewal or extension of the contract.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Distribution and Differentiation of Population Within Metropolitan Areas

Contractor: University of Michigan
Money Allocated: $38,193

Objectives: This study will analyze 1970 U.S. census data on tapes for 20-25 SMSAs and compare these with census materials for 4 Canadian metropolitan areas to determine: (1) the degree of residential segregation of different socio-economic groups; (2) the degree of segregation of families in different stages of the life cycle; (3) the pattern of segregation of housing of different types and ages; (4) the relation of housing type to socio-economic characteristics of occupants; and (5) variations in population distribution in different metropolitan areas.

The distribution and differentiation of population in cities is of major importance, but to date there have been few empirically based studies on this topic. This study will provide a framework for considering the similarities and dissimilarities of 25 cities, which is a sufficient number of the Standard Metropolitan Statistical Areas to give meaningful results. In the process it will develop generalized analytical tools for the study of other groups of cities. This research is more than just a study of segregation patterns and looks at a number of associated factors including socio-economic groups and structures and life cycles in relation to housing.

Significance to Biomedical Research and Program of the Institute: This study will have a great deal of explanatory power concerning the population dynamics of our urban communities, and the Canadian data will offer an interesting comparison. Urban planners should find the project's results useful. Thus, this research is extremely relevant to the Institute's goals.

Proposed Course: This is the first of three years' funding. Approved funding for FY 1972 is $30,548 and for FY 1973 is $42,405, making a three-year total of $111,146

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Rates and Consequences of Population Change: The Growth and Decline of Counties in the Mountain Region

Contractor: Colorado State University (Fort Collins)
Money Allocated: $33,268

Objectives: This study will describe the population changes that have occurred in the Mountain Region (8 States, 279 counties stretching from Montana to Arizona) during the period 1950-1970. Almost one-half of these counties lost population between 1950 and 1960 and more than one-half declined in population during the decade 1960-1970. The main interest centers on the consequences of population change--consequences to public finances, retail activity, housing utilization, and agricultural activity.

Censuses of population, governments, businesses, housing and agriculture, and other published and unpublished pieces of information (available from State and local sources) will be used. Components of change as well as some indices of social welfare, such as doctors per capita, use of health services or school retardation, and grade continuation rates will also be explored to clarify some of the relationships and problems being investigated.

Significance to Biomedical Research and Program of the Institute: This study is both worthwhile and program relevant. Depopulation is one of the more important, yet neglected, aspects of the population problem in the United States. Most of this Nation's counties either lost population or experienced net outmigration in the last two decades, and very large geographic areas are coming to have sparse populations. Few studies have investigated the process or the consequences of such population losses.

Proposed Course: This is the first of two years' funding. Approved funding for FY 1972 is $52,213, making a two-year total of $85,481.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Economic Analysis of Migration from Rural Eastern Kentucky to Selected Urban Centers

Contractor: University of Kentucky
Money Allocated: $72,923 (FY 1970)

Objectives: The purpose is to measure the impact of migration in economic terms on individual donor and recipient local governments and the Federal Government. It will seek to measure and analyze the cost and returns of population relocations through migration from eastern Kentucky to recipient metropolitan areas in Kentucky and Ohio and to isolate the major socio-economic variables giving impetus to this movement. It will include analyses of both initial migration and return migration and the marginal cost of providing selected public services in high density neighborhoods in the cities studied.

Significance to Biomedical Research and Program of the Institute: The evaluation of the consequences of migration is especially relevant to the program of the Center for Population Research, which has as one of its emphases the study of the consequences of population growth and change. It will provide information of concern to the Public Health Service in that it will document the cost to both donor and recipient communities of migration within the United States.

Proposed Course: This contract was initiated in FY 1970, and a funding supplement is under consideration.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Demographic Transition Without Urbanization: Rural Fertility Reduction in the Southern Philippines

Contractor: University of Colorado
Money Allocated: $21,139 (FY 1970)

Objectives: The purpose is to determine the extent to which fertility of migrants to essentially rural areas may be affected by the type of economic and social arrangement in the new area. The investigator will study four communities of migrants in the Philippines, two of which can be categorized as innovative (i.e., encouraging the development of new forms of social interaction motivated by the desire for profit) and two that may be categorized as replicative (i.e., similar in social structure and way of life to the area from which the migrants came) to determine to what extent fertility in the former localities or communities is lower than in the latter type communities.

Significance to Biomedical Research and Program of the Institute: A finding that fertility can be lowered without urbanization, and the specification of some of the conditions under which this happens, would be of immense significance to the formulation of population policies throughout the world.

Proposed Course: This study, begun in fiscal 1969, is expected to be completed in fiscal 1971.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Population Policy-Related Attitudes and Values
Contractor: Carolina Population Center
Money Allocated: $29,821

Objectives: This study will assess by several methods the attitudes toward specific and general population policies in the area of fertility and population growth, and the salient values which are related to, or involved in, the proposed policies, with a view toward policy development and application. More specifically, the investigators will: (1) determine the knowledge and attitude of three groups--(a) legislators, (b) key leadership groups, and (c) constituent groups, toward population policies; (2) measure the underlying value structure of the attitudes; (3) explore the similarities among the attitudes and values of the three groups studied; and (4) generate suggested programs for developing and expediting population policies.

The study will develop accurate and valid methods of (a) measuring knowledge, attitudes, and values of the three groups; (b) identifying members of these groups; and (c) comparing the three groups on relevant measures.

Significance to Biomedical Research and Program of the Institute: This research will make a technical and methodological contribution to the study of attitudes held by sub-groups toward population values and policies. As such it is needed research in a very significant area that is highly relevant to the Institute's interests regarding the development of population policies.

Proposed Course: It is anticipated that this project for a pilot study, including planning and instrument development, will be completed within fiscal 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Effect of Income Payments to Reduce or Increase Natality

Contractor: University of Illinois
Money Allocated: $50,217

Objectives: This study will explore the effect of income on fertility in two different studies: (1) by asking suppositious questions in a sample survey administered to a regional sample of the U.S. population concerning the effect on personal fertility of government payments for more or fewer children; and (2) by studying the relationship between income and fertility using the 1 in 1,000 tape from the 1960 Census, using multiple regression techniques within fine subclassifications and interpreting results with the aid of demand theory.

Significance to Biomedical Research and Program of the Institute: The relationship between income and fertility comprises a critical area of research and thus is highly relevant to the Institute's program.

Proposed Course: The contract, initiated in fiscal 1971, is anticipated to run for two years.

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Population and Reproduction Grants Branch
Center for Population Research

The Population and Reproduction Grants Branch has the responsibility of administering all research and training grants submitted to the National Institutes of Health and assigned to the National Institute of Child Health and Human Development in the biological, clinical, biomedical and social (behavioral) areas relating to population problems and aimed at gaining a fuller understanding of normal and abnormal reproductive phenomena and early developmental processes in animals and human beings. It is concerned with all aspects of population fertility and infertility, particularly studies likely to yield new information and methods for the development of safer, more acceptable contraceptives, as well as with the training of young investigators to accelerate and broaden the research effort. The mission of the Branch was broadened this year with the initiation of the new Population Research Center Award Program.

As of January 31, 1971, grant funding of $11.7 million supported 193 research grants, 29 training grants, 37 fellowships and 18 research career development awards. Approximately 83 percent of these funds support research in the area of reproductive biology and contraceptive development, 9 percent in animal behavior and ecology and 8 percent in social aspects of population research.

The interests and responsibilities of the biomedical program of the Branch range from the origin and formation of the germ cell to the initial stages of uterine implantation, including research in neuro-endocrine controls of reproduction; normal and abnormal reproductive behavior; units of inheritance and sex determination, environmental factors affecting reproductive process, developmental biology of neural and endocrine organs, evaluation and improvement of existing contraceptive methods and development of new methods of fertility regulation, and problems of infertility.

A major emphasis of the grants program on the behavioral-social science aspects of population research has been to encourage and stimulate research proposals from increasing numbers of investigators in a variety of disciplines. Traditionally, sociologists-demographers have performed most of the population research. Recognizing the need for more interdisciplinary research as well as for involving other social sciences to a greater degree, economists, psychologists, anthropologists, geographers, and political scientists (psychiatrists to a lesser extent) have been stimulated to develop population research interests and activities. Thus far, some 500 psychologists have requested research application packets and about 60 have submitted proposals.

The behavioral-social science population research grant program has exhibited considerable increases in the number of proposals, as follows (number per Council): In 1969: March - 4; June - 8; November - 5. In 1970: March - 11; June - 37; November - 44. In 1971: March - 55; June - 56. The number of

proposals has increased to the point that the Division of Research Grants is planning to replace the Special Study Section (Population) with a regular Study Section.

Depicting the substantive areas of current grant proposals in the social sciences gives an overview of the proposals. The following table classifies the research grant proposals submitted for the November 1970 and the March and June 1971 Councils:

Substantive Areas of Research Proposals

Area	No. Proposals	Per Cent
Fertility		
Determinants of fertility	(48)	
Family planning	(25)	
Family size preference	(4)	
Contraception	(8)	
Abortion	(18)	
Sterilization	(1)	
Fertility - Total	104	66%
Antecedents and Consequences-population structure, distribution, change	22	14%
Family structure, sex behavior, childrearing and development	27	17%
Population policy	4	3%
Total	157	100%

This table shows that the proposals are concentrated in the fertility area. The number of proposals to do research on abortion indicates that the workshops and other factors have stimulated interest in this field. The remaining three classifications of research areas have received far too little attention.

The mission of the training grants program relates directly to the major aspects of basic reproductive biology and contraceptive development, as well as the behavioral-social sciences. Specifically, it fosters and supports research training in the following areas: reproductive physiology and behavior and their disorders; the behavioral and biomedical aspects of fertility; the biological, economic and psychosocial consequences of fertility regulation and experience, and their interaction with other demographic variables; the development of population policy; normal and abnormal development of the preimplantation zygote; and implantation and

placental induction.

By the end of Fiscal Year 1971, it is expected that approximately 140 pre- and postdoctoral trainees will be in training; approximately 75 percent are supported on a full-time basis. From data available from progress reports on about 100 past trainees, it is estimated that 92 percent of full-time trainees go into teaching and research, 4 percent receive commissions in the Armed forces, 3 percent enter clinical practice and 1 percent accept positions in industry.

During Fiscal Year 1971, 25 training grant applications were reviewed by the Population Research Training Committee, of which Dr. Bengt Liljeroot is executive secretary. Of these, 18 were new applications and 7 were competing renewals. A total of $2.49 million was requested by the applicants. The committee recommended approval for 12 new applications for $1.10 million; all seven competing renewals were recommended for continuation for $0.65 million. Because of limited resources, only one new training grant and 2 renewals were awarded for $0.34 million. Fifteen non-competing renewals were funded for $1.12 million. Of the 18 training grants funded by the Center for Population Research during Fiscal Year 1971, eight were for training in the biological sciences, three in behavioral and social sciences, and seven for research training in a clinical department.

The Center for Population Research also supports research training through a Postdoctoral and Special Fellowship program and the Research Career Development Award. During Fiscal Year 1971, $764,000 was available for these awards. Approximately $620,000 was used for previous obligations including 18 Research Career Development Awards and one Research Career Award. The remaining funds were used for six new Research Career Development Awards and three new Postdoctoral fellowships.

During this year, several staff changes have occurred. Dr. James O'Donnell, Reproduction & Population Branch Chief, resigned this position in December, 1970, and moved to the Division of Research Resources. Mr. Arthur Campbell, deputy director, Center for Population Research, assumed responsibilities as Acting Branch Chief until Dr. Everett Wilson, a reproductive biologist from Sam Houston University in Texas, joins the Center in June as Branch Chief. Dr. Suzanne Hurd has been assigned to the staff as a scientist administrator where she shares the work and responsibilities with Dr. Allyn Waterman in the biomedical area.

Six scientists enrolled in the Grants Associate Program, NIH, spent three to six weeks in the Branch during this year: Drs. Robert Conant, Lawrence Fisher, Richard Gaufin, Zora Griffo, Robert Leyton and Bernard Talbot. They participated in the daily work of the branch and completed a program assignment designed to provide them experience for a career in health science administration.

On July 1, 1970, the Center for Population Research, announced a new program to establish Population Research Centers in order to provide core support and new program development for non-profit organizations conducting research the training relevant to population problems. This program represents a

commitment by NIH to the long-term support of institutions with demonstrated ability to conduct research and research training in areas that seem likely to hasten progress toward solving problems related to human population growth, structure, and distribution. Establishment of Population Research Centers would facilitate multidisciplinary communication and effort in the population field, give visibility to the research area, and provide administrative support and a physical environment conducive to scientific productivity.

In response to published announcements of this new program the Center for Population Research received over 300 letters of inquiry this year. By March 1970, there were 33 letters of intent with accompanying visits of representatives from the interested institution, resulting in formal submission of six Centers applications for the March 1971 Council and four for the June 1971 Council. Following discussions with program staff, five institutions were encouraged to develop program project applications rather than Center applications; one institution submitted such an application which will be considered at June Council.

Program Project grants, in contrast to Population Research Center Grants, provide research support for the investigators. During this year the Program funded seven program projects; one of these was a new award. These awards encompass all aspects of population research, both clinical and non-clinical.

The Population and Reproduction Grants Branch has administered as one aspect of its program the development of conferences, symposia and workshops to assess the present state of knowledge of selected areas of basic reproductive biology deemed particularly pertinent to future contraceptive development, as well as to identify areas where imaginative approaches may accelerate research development and progress. A further purpose is to identify and encourage investigators who may not now be actively working in the field so that interdisciplinary exchange may be effected and rapid progress may be made in solving the complex problems of basic human reproduction.

During this year a publication "The Biology of the Blastocyst", edited by Dr. Richard Blandau, presents the result of a symposium in reproductive physiology organized by Dr. Blandau and held in July, 1969, at Lake wilderness, the postgraduate training center of the University of Washington. The book combines a systematic review of the present state of blastocyst biology and knowledge about its environment and implantation, along with the most recent experimental and methodological developments and suggestions for future research. It is a companion volume to other symposia, held in previous years; important topics that have been discussed previously include: fetal homeostasis, comparative reproductive failure, the mammalian oviduct, etc.

In October, 1970, a symposium on oogenesis was organized and directed by Professor John D. Biggers, D. Sc., Ph.D., and Allen W. Schuetz, Ph.D., of the Department of Population Dynamics, The Johns Hopkins University. The results of the symposium are currently being prepared for publication.

Another topic of interest to the Branch was the subject of a symposium with

accompanying workshops organized by Noland K. Van Demark, Ph.D., Department of Dairy Science, Ohio State University, entitled, "The Environment and Reproductive Processes" and held in conjunction with the annual meeting of the Society for the Study of Reproduction; this symposium continues a series supported by the NICHD. The proceedings were published in the journal of the Society. The purpose was to explore with the participants the importance of the physical and biotic environment to successful reproduction, to demonstrate the role of internal rhythms as an adaptive mechanism utilized by an organism to accommodate itself to an ever-changing environment and to emphasize olfaction as one type of sensory input affecting reproduction.

The second workshop on abortion entitled "Abortion, Obtained and Denied: Research Approaches" was held in December, 1969, jointly sponsored by NICHD and NIMH. A full report of this workshop is being published as a monograph by The Population Council, probably in May or June, 1971. A summary report appeared in Studies in Family Planning, May, 1970.

A workshop entitled "Research Approaches to Sex Education, Contraception, Family Planning and Morality" was held in September, 1970, sponsored by NICHD, organized and chaired by Sidney H. Newman. Its primary objectives were (1) to explore the relationships among the fields of sex education, contraception, and family planning, as well as their common moral issues, and (2) to explore avenues of potential future inquiry in these fields.

Research Excerpts

Beyond the need for goal-oriented research associated with population problems arising with the significant expansion of world population there is an undisputed need for continued support of fundamental investigations which will furnish basic information concerning reproduction. Studies at the biochemical, genetic, physical, physiological, behavioral, and early developmental levels in both the normal and the pathologic, the *in vivo* and the *in vitro*, the vertebrate and the invertebrate, are continuing to help towards an understanding and appreciation of human development. Support for over 190 grants has enabled basic biomedical-behavioral scientists throughout the nation to identify their research interests in basic reproductive biology with the mission of the Center. The discovery of new, safer, more acceptable methods of regulating family size will require the cooperative efforts of biomedical and social scientists from many different disciplines including: biology, chemistry, physics, bioengineering, sociology and psychology. The problems associated with human infertility continue to be of concern; failure to have children can be as disturbing to healthy family life as too many children and/or too closely spaced children.

Goals and conditions of population control. Dr. Kingsley Davis, University of California at Berkeley, is focusing on social and demographic research bearing on population policy. Six publications and a Ph.D. thesis were completed during the first year of the project. Dr. Arriaga completed a study of the effects of existing population policies on social development in Latin America. An analysis has been made of family formation in Eastern Europe, including parts of the Soviet Union.

Family and Fertility Among Mexican-Americans. Doctors Bean, Browning, and Bradshaw, University of Texas, are making considerable progress in their study of Mexican-American fertility. They are producing some interesting findings, but indicate that these are still tentative. For example, they find that only 10% of the Mexican-American couples samples have not used any form of contraception. About two-thirds of the women have at one time or another taken the pill. The more recent birth cohorts are turning increasingly to contraception. The investigators are still analyzing data on level of completed fertility; impace of child spacing on family economic position; the relationship between socioeconomic status, ethnicity, and fertility; and on religiosity and fertility.

Fertility and family planning in Taiwan. This study of fertility and family planning in Taiwan, being conducted by Dr. Ronald Freedman and associates, University of Michigan, is in its fourth year. The investigators have studied many aspects of changing fertility levels and their determinants in a rapidly developing society. A critical part of the study was observing the growth and influence of a mass family planning program. More than 30 articles and a book on bio-social factors affecting fertility have been published. The data have been the basis for eight doctoral dissertations. The study has resulted in a unique body of time series data on the social, economic, and demographic determinants of fertility and family planning. Both the program in Taiwan and analyses of it have been closely followed and have influenced family planning programs and methods for their evaluation in many parts of the world.

Measurement methods for population change. This study by Dr. Forrest Linder and colleagues, University of North Carolina, is in its third year. Progress was made in the development of a general comprehensive theory and initiating a series of systems analyses of specific measurement systems. The theory of randomized response methods was extended and applied. Other studies were on mathematical and computer models related to conception time, coital frequency, fecundability, birth intervals and family building models. Eighteen papers have been prepared, 2 doctoral theses completed, and five doctoral theses are near completion.

Biosynthesis of Steroids. Researchers are investigating the relationships between the fundamental biochemical facts and the complex system of reproduction. Coordination in this regulatory system involves both mechanisms for control proceeding from the central nervous system outward and multiple feedback loops which serve to inform the central regulatory sites of the status of the peripheral organs so that appropriate adjustments can be made in the total process. The messengers in this regulatory process and the informational signals in the feedback loops are for the most part hormones, polypeptide and steroid. Dr. Ahren, University of Gotenborg, Sweden, has demonstrated that one of the effects of FSH is to increase the uptake of amino acids by the ovary. This effect, however, is blocked by puromycin, suggesting that FSH acts primarily on the genetic-protein synthesizing machinery. FSH, therefore, makes possible the biosynthesis of steroid hormones by the gonads but does not of itself regulate the process.

The effect of LH on the biosynthesis of steroids is under study by Dr.

Hagerman, University of Colorado, Dr. McKerns, Harvard University, and Dr. Armstrong, University of Western Ontario. The primary effect of LH in the corpus luteum is to accelerate the biosynthetic pathway between cholesterol and pregnenolone. It is apparently not mediated by way of the protein synthesizing apparatus but appears to involve some sort of an allosteric transformation of preexisting enzyme which becomes more active in the presence of the gonadotropin. Dr. Savard, University of Miami, has evidence to indicate that a second messenger, cyclic adenylic acid, is involved in the reaction.

Dr. Christensen and Dr. Gillim at Stanford University are using cytochemical and histochemical techniques and autoradiography at the electron microscope level to allow a more detailed localization within the organelles of the enzymes involved in steroid biosynthesis. Only then will some of the questions of concern in modern cell biology be answered such as transport, mechanism of action, mechanism of secretion, and localization.

Sperm Migration

Among the unanswered questions regarding human reproduction, none is more important than, "How do sperms reach the egg?" Dr. Moghissi, Wayne State University, has shown that migration through the cervical canal is influenced by proteolytic activity of seminal plasma and spermatozoa, phalanx formation due to the difference in surface tension between semen and cervical mucus, and possibly the orientation of strands of cervical mucus mucoid. Cervical mucus functions not only as a sperm reservoir but also as a biologic filter where dead, abnormal or unfit spermatozoa are retained and prevented from reaching the uterine cavity. Contractions of tubal musculature, ciliary activity of the endosalpinx and fluid currents within the oviduct seem to be responsible for sperm transport to the site of fertilization according to the observations of Dr. Blandau at the University of Washington. Although the primary stimulus for such a precise and dynamic physiologic process is not known, recent data suggests that oxytocin and prostaglandins, in addition to estrogen priming, are likely responsible factors.

Drs. Iwamatsu and Chang at the Worcester Foundation for Experimental Biology are studying capacitation and have clearly demonstrated the requirement of capacitation of mouse sperm before these are capable of penetrating the eggs. The time required for capacitation of mouse sperm is about one hour in contrast to 2-4 hours in the hamster and 6 hours in the rat. Dr. C. E. Hamner and colleagues at the University of Virginia reported data indicating that cat spermatozoa require capacitation for successful fertilization. It has yet to be demonstrated, however, for man or the sub-human primates.

Millions of spermatozoa gain entry into the female genital tract with each ejaculation. Phagocytosis seems to be the chief means of disposal of surplus sperm. Intense leukocytic activity with engulfment of spermatozoa by uterine glands, endosalpinx and surrounding tissues have been observed within the utero-tubal segment. The immunologic consequence of sperm phagocytosis may be the production of antisperm immune anti-body and subsequent infertility.

Acrosome. Knowledge of the enzyme type and content in the acrosome region of the spermatozoan head has been sought vigorously, since these enzymes aid the spermatozoan in penetrating the cells and membrane enclosing the mammalian egg. Extracts contain proleolytic and hyaluronidase activities. Drs. Penelope Gaddum and Richard J. Blandau, School of Medicine, University of Washington, have recently applied a technique which for the first time provides a direct, visual demonstration of the presence of a proteolytic enzyme from individual sperm heads on India ink-gelatinized plates. The characteristics of the enzyme noted so far suggest that it may be the "trypsin-like" enzyme identified by others as the primary agent effecting dissolution of the egg membrane during fertilization. If true, this could be a useful method for studying the potential of different populations of sperm to effect penetration of the egg membrane.

Prostaglandins. The ubiquitous fatty acid prostaglandins--or "prostinoids"--reported in human and animal tissues have a wide range of pharmacologic roles as intracellular metabolic regulators that block the action of a number of hormones. Of particular interest to reproductive biologists are those that have been shown to have potential medical use in fertility control: induction of labor, therapeutic abortion, contraception, and male sterility. They could be effective as a once-a-month or morning after contraceptive by inducing a menstrual period and thus avoid the continuous prolonged dosage of current birth control compounds.

A number of Center-supported scientists are studying these compounds. Dr. John M. Marsh, University of Miami, has found that PGE_2 brings about an increase in cyclic AMP by activating the adenyl cyclase rather than by inhibiting the phosphodiesterase system. He suggests that this compound stimulates steroidogenesis (progestin synthesis) in incubating slices of corpora lutea via the mediation of cyclic AMP. The functional significance of this effect of PGE_2 in corpora lutea is unknown; it could be a mediator of the action of LH, acting between LH and its effect on adenyl cyclase. If this were true, it would require that LH increase the concentration of PGE_2 or other prostaglandins in the luteal tissue.

The function of prostaglandins in reproduction has been reviewed and discussed in a recent article by Dr. Kamran S. Moghissi and Charles P. Murray, Wayne State University School of Medicine. Although produced in the male system in large quantities, there appears to be no evidence that prostaglandins play a role in the human male reproductive tract or have and direct action on sperm movement and survival in the female system other than by altering uterine activity. Evidence seems inconclusive that prostaglandin-deficient semen is associated with infertility. Prostaglandins are absorbed readily from the vagina, and act on smooth muscle. Evidence for the role of prostaglandins in regulating the life span of the corpus luteum in the nonpregnant state is tenuous, but they may affect its survival by action on ovarian blood flow. Studies currently being vigorously pursued by many scientists represent the most exciting development in recent research in the reproduction area.

Another substance found in semen has been reported by Drs. Matthew Freund and William Ventura, New York Medical College, and has been shown to have a

stimulatory effect on all parts of the female reproductive tract. The investigators have named this factor "spasmogen". Although it is apparently distinct from prostaglandins, it could be a carrier molecule to which such compounds are bound.

Oviductal fluid. The composition and characteristics of the luminal fluid of the primate continue to receive investigative attention. Recently Dr. Luigi Mastroianni and colleagues at the University of Pennsylvania School of Medicine have reported that a study of the electrophoretic patterns of monkey tubal fluid collected continuously before, during and after ovulation has revealed the appearance of a special protein following ovulation, indicating an active secretion in the monkey oviduct.

Dr. Kamran S. Moghissi, Wayne State University School of Medicine, has described a method for collecting human tubal fluid. He has reported that it contains approximately half as much protein as does serum, but with a distribution of major protein fractions similar to that in serum. A total of 15 serum type proteins was identified, and their patterns suggested both transudation and active secretion. Dr. Moghissi, also reports the presence of a specific tubal β-glyco protein (X-glycoprotein), absent in serum and apparently produced by the fallopian tube.

Protein binding of steroids in rabbit oviductal fluid, as influenced by hormonal status, has interested Dr. Mastroianni and his colleagues. Oviductal protein patterns failed to show the presence of the postalbumin protein observed in rabbit uterine fluid after ovulation. In the castrate, progestrone, or progesterone plus estradiol, induced the appearance of this protein in uterine fluid, but not in oviductal fluid. Estradiol had no effect. This protein displayed a pattern similar to "blastokinin" of Krishnan and Daniel, and the "uteroglobin" of Beier.

Ovulation. Progestational agents are known to interfere with ovulation, a principle exploited in the form of the oral contraceptive pill. The hypothalamus normally releases ovulating-hormone releasing factors upon neurogenic activation. Dr. S. M. McCann, Southwestern Medical School, Dallas has reported on evidence indicating that dopamine may be the synthetic transmitter involved in the release of these factors from neurosecretory neurons. Although the precise chemical nature of the gonadotropin-releasing factors remains elusive, they have been prepared in highly purified form. It is thought that they are small peptides.

The releasing factors, in turn, are postulated to be transported to the pituitary, whereupon release of ovulating hormone(s) into the systemic circulation is effected. Work by Dr. Hilliard at the University of California at Los Angeles and Dr. Spies at the Delta Regional Primate Center has suggested that the progestogens exert their effect by inhibiting the ability of the pituitary to secrete ovulating hormone in response to its releasing factor. Recent evidence by Dr. Spies indicates that in the rat and in the rabbit, progesterone probably blocks ovulation by a direct action on the cells of the adenohypophysis. Dr. Lisk of Princeton University, however, reports that in the rat, progesterone appears to inhibit ovulation by acting at the level of the nervous system. Thus, although several

investigators are exploring this critical question, the point at which progesterone acts in the sequence of events culminating in the release of ovulating hormone is still equivocal.

Freeze Preservation of Human Sperm

Large differences exist between species with respect to the ability of their spermatozoa to survive freeze preservation techniques. Dr. Behrman of the University of Michigan Medical Center is studying cryogenic requirements for human male gametes as technologic advances in semen banking will aid in infertility studies. In addition, clinicians have displayed considerable interest in the idea that large psychological as well as practical advantages would ensue if family planning were conducted on the basis of vasectomy preceded by the preservation through freezing and storage of a sufficient number of the individual's semen specimens to insure that he may have in the future as many children as are desired.

From a clinical standpoint it has been demonstrated that human spermatozoa can be preserved in a functional condition for a short period of time. Conception with this technique requires almost the same number of inseminations as when fresh sperm are used but the efficacy, or conception rate, is definitely lower when using frozen sperm. Although the fertilizing capacity of semen from normal donors is reported to be altered after having undergone freezing preservation treatment for any length of time, this aberrance is not due to lack of embryonic losses, failure of capacitation or lack of transport on the part of aged spermatozoa. Furthermore, there is no evidence indicating any increases in abortion, in congenital defects, or in any pathology as a result of using frozen semen.

Medical Effects of Contraceptives

Agents which cause chromosome breakage and rearrangement are of unique importance because of the potential they may possess for the production of teratology and neoplasia. Estrogens and progestins, currently used in the area of fertility control by women in the child bearing years, deserve special attention regarding the potential of cell damage. Dr. Stenchever of Case Western University evaluated various concentrations of five progestins and three estrogens, constituents of the widely used contraceptive pills, in 72-hour human leukocyte cultures for their effects on chromosomal integrity. No significant chromosomal abnormalities were found in any concentration of any substance studied.

NICHD Annual Report

July 1, 1970 through June 30, 1971
Program Liaison Branch
Center For Population Research

The Interagency Committee on Population Research (ICPR) has been established as a formal interagency committee chaired by the Director of CPR with the executive secretary (Rolf Versteeg) and staff support coming from the Program Liaison Branch. An inventory of ongoing Federal research activities in the population field was updated to July, 1970 and published by the Center for Population Research under the title "The Federal Program in Population Research". The ICPR is now reviewing the results of the inventory and preparing a report and recommendations to the Deputy Assistant Secretary for Population Affairs to be completed by June 30, 1971.

The improvement of scientific and technical communications in the population field continued as a major concern to the Program Liaison Branch. A number of consortium meetings brought together some of the major producers and disseminators of scientific and technical information both inside and outside of government, under the aegis of the Program Liaison Branch. The Program Liaison Branch continues to provide the executive secretariat and staff support for this semi-formalized scientific and technical information network until other arrangements can be made. Several briefings were held for the Deputy Assistant Secretary for Population Affairs and written recommendations were made to him concerning the desirability of transferring to his office responsibility for governmentwide coordination of scientific and technical communications concerned with family planning and population matters.

Related to communication concerns, the Program Liaison Branch (Mr. Versteeg) has been actively assisting in the development of a formalized Association of Population Libraries and Information Centers and exploring the possibility of affiliation with the Population Association of America.

The Program Liaison Branch has been heavily engaged since December, 1970 in an inventory and review of ongoing Federal Research and Development activities exploring the impact of population factors on environmental quality. The Program Liaison Branch provides the chairmanship (Dr. Hilmar) and staff support for the special sub-committee on population matters which is preparing its report for inclusion in the larger report of the FCST Ad Hoc Committee on Environmental Quality Research and Development.

Program Liaison Branch staff (Mr. Versteeg) made studies and proposals for modifying the Department patent rights policy applicable to the contraceptive development program of CPR. This has involved discussions with program and legal staff in an effort to recommend ways of developing a variety of new and improved methods of fertility regulation through mutually productive relationships between Government and industry.

The Program Liaison Branch (Mr. Versteeg) had the major administrative and coordinating responsibility for the preparation of the CPR five year plan requested by Dr. Louis Hellman as required under the provisions of the Family Planning Services and Population Research Act of 1970.

As a part of its coordinative and informational functions on behalf of CPR the Program Liaison Branch maintains a widening network of contacts with such outside agencies as the Human Life Foundation, Planned Parenthood-World Population and the Pan American Health Organization as well as with a number of colleges, universities and other organizations seeking to develop curricula dealing with population, environmental pollution and ecology in an interdisciplinary manner.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Office of Associate Director for Program Services
Extramural Activities

SUMMARY

An increase in the contract management activities and the realignment of the mission of the Program Statistics Branch and the Scientific Information Centers Branch were major factors in the Institute's Office of Program Services during the year. A total of 210 contracts which have a value of about $16.6 million have been processed. This more than doubles the $7.7 million processed in Fiscal Year 1970. At the same time while there was a small decline in the number of research grants processed in the Grants and Contract Management Branch (54), there was an increase of $4.4 million over the 1970 total, for a 1971 total of $46.3 million. Training grants remained about stationary in 1970, 118 awards were made for a total value of about $10.0 million. The total portfolio of research and research training grants, research fellowships, research career and research career development awards and research contracts was about $76.8 million in Fiscal Year 1971. This represents an increase of $16.0 million over Fiscal Year 1970.

The termination of publication of the two experimental abstract journals, "Adult Development and Aging" and "Population and Reproduction" provided the Institute the unusual opportunity of evaluating the priorities in Program Statistics and the Scientific Information Centers Branch. This resulted in a reorganization and the title of the Scientific Information Centers Branch was changed to Scientific Information and Publication Branch with greater emphasis on the management of scientific publication reference services. The Reference Service Section acquires, catalogues and maintains library material for use by NICHD staff. It also compiles demand and recurring bibliographies and maintains an Interlibrary Loan Service. Unfortunately, space problems necessitates the section be divided into two geographic locations--in Building 31 and in the Westwood Building. This has complicated both the distribution and easy access of materials. The Section handled the purchase of over 1,300 books and filled almost 1,400 interlibrary loan requests. The Scientific Publication Section provides central service for editing, composing and designing Institute publications. It also arranges for the printing, storage and distribution of published material.

The Program Statistics Branch became the Program Statistics and Analysis Branch with added emphasis on research analyses and information systems. The Research Analysis Section of this Branch is responsible for the printing of grant and contract summaries including research progress and the analysis of research accomplishments of NICHD grantees. The Information System Section is responsible for the development of systems for the storage and retrieval of data relevant to the Institute's programs and for the coordination of computer activities within NICHD and of NIH and external facilities as required.

The Scientific Conference Branch in addition to assisting in the planning and arranging of 21 conferences under the direct sponsorship of NICHD also assisted in the management of eight NICHD Advisory Committees which includes the National Advisory Child Health and Human Development Council. Of the approximately 525 scientists who participated in the 21 meetings almost half of them were first time participants in Institute conferences.

A major problem which affected the working relationship with the rest of the Institute was created when the Scientific Conference Branch, Scientific Information and Publication Branch, and Program Statistics and Analysis Branch were transferred to the Westwood Building in the fall of 1970. While every effort is being made within the Office of Program Services to compensate for this move the results have not been satisfactory and the effectiveness of the three branches has suffered. The reduction of face-to-face discussion between Westwood staff and those in Building 31 has been particularly disturbing. Remedial factors include the assignment of Branch staff to particular program liaison activities which includes their attendance at staff meetings held in Building 31. Conversely, Office of Program Services' Branch meetings are now held routinely in the Westwood Building.

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July 1, 1970 through June 30, 1971
Grants and Contracts Management Branch

SUMMARY

During Fiscal Year 1971, the Institute should support about 1,273 research and research training grants, research fellowships, research career and research career development awards and research contracts at approximately $76.8 million. This would represent a decrease of less than 1% in numbers of projects (8) and an increase of 26% in dollars ($16.0 million) from the Fiscal Year 1970 portfolio.

The Institute continued to use a decile system as the basis for funding approved research grants. The decile system, based on Council priority scores for Fiscal Years 1969 and 1970, was used as the basis for funding approved research training grants. The latter funding system was further modified by selecting 50% of training grant competing funds for grants submitted from clinical departments and 25% each of these funds for grants submitted from basic biological and behavioral sciences departments. The priority score was essentially the basis for funding the research fellowship, career award, and research contract programs, although selected adjustments in funding order were permitted because of program relevance.

Negotiation of Research and Training Grants

Noncompeting research grants were not negotiated during Fiscal Year 1971. All competing research grants were negotiated at an average rate of 6%. Through these negotiations we expect to acquire an additional $0.7 million which will enable us to fund about 10 additional grants.

Noncompeting training grants were negotiated at an average rate of 6%. Competing grants were negotiated at an average rate of 6% until the last quarter of the fiscal year when the rate was changed to 10%. The negotiations will result in a savings of approximately $.7 million which will enable us to fund 10 additional training grants.

Research Grants

During Fiscal Year 1971, we expect to award 745 regular research grants in the amount of $42.3, a decrease of 54 grants and an increase of $4.4 million over Fiscal Year 1970. The total consists of approximately 530 noncompeting grants for $30.4 million and 215 competing awards for $11.9 million. In our Special Research Program we awarded 9 Mental Retardation Center grants for $3.9 million. We transferred $3.9 million to the Division of Research and Resources for the General Research Support Grant Program. We will award $0.1 million for scientific evaluation grants.

Training Grants

During Fiscal Year 1971 we expect to award 118 training grants for $10.0 million, a decrease of 13 grants and an increase of $.7 million over Fiscal Year 1970. The total consists of 92 noncompeting grants for $7.4 million and 26 competing grants for $2.6 million. We will obligate all of the $0.1 million available for scientific evaluation grants.

Fellowships

We expect to award approximately 75 postdoctoral and special fellowships in the amount of $0.8 million. This is a decrease of 11 awards and $0.1 million from Fiscal Year 1970.

Research Career Awards and Research Career Development Awards

During Fiscal Year 1971 we expect to award 116 research career and research career development awards in the amount of $3.0 million. The total consists of 7 research career awards for $0.2 million and 109 research career development awards for $2.8 million. This represents an increase of 3 awards and $0.1 million over Fiscal Year 1970.

Research Contracts

During Fiscal Year 1971 we expect to process 210 contracts and reimbursable agreements for approximately $16.6 million. This will consist of approximately 175 research contracts and reimbursable agreements in the amount of $13.1 million for the Center for Population Research Program and approximately 35 research contracts in the amount of #3.5 million for the remainder of the Institute's programs. The latter will include support primarily for the Early Child Development and Nutrition programs identified as new initiatives in the President's Budget for Fiscal Year 1971.

The Fiscal Year 1971 contract program will represent a net increase of 67 contracts and reimbursable agreements and $8.9 million over the Fiscal Year 1970 program.

SUMMARY OF INSTITUTE SUPPORTED PROJECTS
(Dollars in Millions)

	1970		1971		Change	
	No.	Amount	No.	Amount	No.	Amount
Research Grants						
Noncompeting	545	27.0	537	33.3	-8	$ + 6.3
Competing	263	13.2	217	13.0	-46	- .2
	808	40.2	754	46.3	-54	$ + 6.1
Training Grants						
Noncompeting	110	7.4	92	7.4	-18	-0-
Competing	21	1.8	26	2.6	+ 5	+ .8
Scientific eval.		.1		.1		-0-
	131	9.3	118	10.1	-13	+ .8
Research Fellowships	86	0.9	75	0.8	-11	- 0.1
Research Career and Research Career Development Awards	113	2.7	116	3.0	+ 3	+ 0.3
Contracts	143	7.7	210	16.6	+ 67	+ 8.9
Grand Total	1,281	$60.8	1,273	$ 76.8	- 8	$16.0

NICHD Annual Report
July 1, 1970 through June 30, 1971
Program Statistics and Analysis Branch

Toward the end of FY 1971, a reorganization within the Office of Program Services resulted in expanded functions for the Program Statistics Branch. The new title of the Branch is the Program Statistics and Analysis Branch, which now has four Sections; Statistical Analysis, Program Analysis, Research Analysis, and Information Systems.

There were no major functional changes in the Statistical Analysis Section, which consists of two units, the Recurring Reports Unit and the Analytical Unit. The former has the responsibility of maintaining the Branch's machine records on grant and contract data and issuing recurring reports from this base. The Analytical Unit is responsible for planning and executing special statistical studies on a broad range of subjects related to the mission of the Institute.

The major functions of the Program Analysis Section include the assignment of grant applications to appropriate program areas within the Institute; the scientific classification of grants, contracts, and intramural projects; and the preparation of analytical reports in research areas pertinent to NICHD programs. New responsibilities assumed by the Branch as a result of the reorganization are the preparation of two annual publications for the Center for Population Research, one a Federal inventory of population research, and the other a current survey of population research. The Inventory covers research grants and contracts in population supported by Federal agencies, whereas the Survey includes all population projects supported by both public and private organizations. The Branch is working closely with the staff of CPR in the preparation of these reports.

One of the new Sections added to the Branch is the Research Analysis Section. The major responsibilities of this Section are the preparation of grant and contract summaries, including research progress, and the analysis of research accomplishments of NICHD grantees. These functions were given a high priority by the Director of the Institute and by program staff.

Another new Section is the Information Systems Section, which is responsible for the development of systems for the storage and retrieval of data relevant to the Institute's programs, and for the coordination of computer activities within NICHD and with NIH and external facilities as required.

As in past years, a considerable amount of the work of the Branch was related to the appropriations process. Examples of jobs done in this area are given below.

Background statistics were provided for use at appropriation hearings, showing that the U.S. ranked 14th among major nations in infant mortality in 1969,

and that the birth rate has been increasing for the last two years, along with other detailed information.

To help in evaluating the NIH training programs, material was provided to the NIH Office of Program Planning and Evaluation on projections of requirements for researchers in the program areas of NICHD, the training of individuals needed to fill these requirements, and anticipated deficits.

In order to determine the best use of additional funds available in NIGMS for genetics research, Institutes were asked for information on their activities in this area. The Branch identified FY 1971 unfunded genetics research grant applications for possible funding by NIGMS. The Branch also prepared material on FY 1970 genetics grants to give an indication of the kinds of genetics studies of interest to this Institute.

In connection with the possibility of increased funding for cancer programs, the Branch worked with intramural staff to identify cancer-related research in NICHD.

To assist the NICHD Budget Office in projections required in the preparation of the Budget, this office provided various types of data, such as a count of trainees under NICHD training grants by academic level, and the conversion of figures on part-time and short-term trainees to a full-time-equivalent basis.

Developments within NICHD also generated work for the Branch.

With the funding of the last of the implementation grants for 12 NICHD mental retardation centers across the country, all of the centers are now in operation, and it is essential to be able to keep track of the research being carried out in these centers. Working with program staff, all NICHD grants supported at the centers, including those now in the mental retardation program and those in other program areas, have been identified, and a system set up to keep this information current and on magnetic tape.

The Branch has been occupied with a number of jobs as a result of the change in the definition of population research to exclude developmental biology, as well as the increased interest in this area. We were heavily involved in the reassignment of FY 1970 CPR grants in developmental biology to other program areas and in the development of criteria for this area. In order to facilitate trend studies, grants in developmental biology in earlier years were also reassigned in accordance with current program area criteria. A new subdivision has been established in PBIM to give visibility to that part of the developmental biology program in the Institute, and work is in process to develop a generally acceptable definition of developmental biology across program areas for use on an Institute-wide basis. An analysis of the effect of the developmental biology transfer on the workloads of the NICHD review committees was made for the

Associate Director for Extramural Programs.

The Branch now has the responsibility for the annual publication on research and training in the Adult Development and Aging Branch, which is used to inform Congressional Committees and the scientific community about the Branch's grant and contract programs. Summaries were prepared for each project covering the purpose, methodology, and progress of the research and sent to the investigators for clearance prior to publication. PSAB was also asked to prepare a historical analysis of the review and funding of research and training applications in the ADA program, and to keep this information updated on an annual basis.

Several papers on accidents and accidental injuries were prepared for use as background material for a conference the Institute is holding on this subject. One was an analysis of current research in this area supported by public and private agencies, both in the U. S. and abroad, with the focus on the behavioral factors which precipitate accidents. Another dealt with the specific activities within DHEW relating to data collection, service programs, and research relevant to accidents. The third was a statistical analysis of existing data on accidents and injuries, with particular emphasis on children and the aged.

A good deal of work has gone into the development of major research subcategories within each program area. These were developed in conjunction with program staff to meet the administrative or analytical needs of individual programs, and are now in use for all of the five program areas.

Several studies, as described below, were made during the year for presentation to the NACHD Council, and key Branch staff met with the Council in closed session to discuss the Branch program and the types of analyses of interest to Council members.

A study on the "Fate of Amended Research Grant Applications in NICHD," pointed out the increase in numbers of amended applications and indicated that, based on NICHD experience, disapproved applications have only about one chance in ten of being funded if resubmitted, while approved but unfunded applications have four chances out of ten, which is a more favorable probability than the average NICHD application enjoys.

As a follow-up to an earlier study on trends in NICHD research training support by type of department, comparable information was obtained on "Departmental Trends in the NICHD Research Grant Program." The former study had shown dramatic changes in the distribution of training support among clinical, biological, and behavioral departments since 1964. It turned out that research grant support showed similar trends, with clinical departments receiving a declining share of funds and behavioral departments an increasing share, but the changes were considerably less pronounced.

"Highlights of the NICHD Grants and Contracts Programs, FY 1964-1970" was a study of trends in various aspects of our extramural programs. It pointed out that the number of projects the Institute is able to support annually has been decreasing since 1967 while the number of applications has been increasing. With NICHD research grant approval rates higher in Counci Year 1970 than at any time in the past six years, funding rates, therefore, dropped to a new low. Other developments included the rapid growth of both the MR Centers program and the research contract program in the Center for Population Research.

With the emergence of the Office of Child Development, work has been initiated to coordinate activities of DHEW in the area of early child development. As a part of this effort, the Branch was asked to identify and classify, within a system developed by OCD, all NICHD research grants in which the primary subjects were children under the age of 6 years. Branch staff also assisted the Office of Program Planning and Evaluation in representing the Institute at meetings of the Interagency Committee on Early Child Development. This group was established to assess the state of the art in this area, and to make recommendations to OMB.

Charts and back-up data were prepared for use in the chart book of the White House Conference on Children and also for that of the White House Conference on Youth. The Chief of the Branch represented NIH first on the Interdepartmental Advisory Committee on the Children's Chart Book, and later on the comparable committee for the chart book on youth. Charts the Branch provided for "Profiles of Children" included such subjects as maternal and infant mortality, pregnancy outcome, abortion, family planning, mental retardation, blindness, childhood cancers, etc. "Profiles of Youth" will be published in June.

A large variety of other special requests for information were filled by Branch staff. Some were related to specific research areas, such as hematology, initiation of labor, vasectomy, rhythm method, pharmacology and toxicology, toxemia of pregnancy, iron deficiency anemia, phenylketonuria, minimal brain damage, aging and the nervous system, sickle cell anemia, CNS malformation and dysfunction, day care programs, etc.

Others dealt with administrative and fiscal aspects of the Institute's programs; such as an analysis of Population and Reproduction grants and contracts by type of grantee institution, a study of trends in the review actions of certain review groups, and a distribution of research grant applications by amount requested and by PHS region.

The Branch continues to issue summary reports of active grants and contracts as well as detailed listings in numeric, geographic, and alphabetic order for the use of Institute staff; and to prepare annual reports for the National Science Foundation, the Secretary's Committee on Mental Retardation, and the Fogarty International Center.

Table 1 summarizes the Institute's portfolio of grants and contracts active as of March 31, 1971. Table 2 provides number and dollar totals on NICHD grants and contracts by mechanism of support for the period FY 1964 through FY 1970. The decline in number of grants supported, which began in 1968, continued in 1970 even though the amount obligated increased. The research contract program showed substantial growth in 1970, due to the availability of funds for population research. Training grants and fellowships, however, decreased in 1970, both numberwise and dollarwise; the fellowship program was reduced to less than half of its 1969 size.

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NICHD Grants and Contracts Active as of March 31, 1971

Grants and Contracts

Mechanism of Support	Number						Funds (thousands)					
	Total	PR	PBIM	GD	ADA	MR	Total	PR	PBIM	GD	ADA	MR
Research Grants	837	199	228	252	59	99	$46,473	$ 9,297	$9,530	$12,824	$4,081	$10,740
Training Grants	a/153	29	30	47	28	18	a/10,913	2,269	2,432	2,767	2,136	1,184
Fellowships	72	31	14	13	5	9	678	272	137	107	62	99
RCP Awards	114	23	31	40	7	13	2,791	487	779	1,031	190	303
Total Grants	a/1,176	282	303	352	99	139	a/60,854	12,325	12,879	16,730	6,469	12,326
Contracts	b/177	b/152	3	8	7	7	b/10,438	b/8,375	122	1,103	329	509
Total Grants and Contracts	a/b/1,353	b/434	306	360	106	146	a/b/71,293	b/20,700	13,001	17,833	6,798	12,835

Research Contracts

Organizational Unit	By Relevant Program		By Organizational Unit	
	Number	Funds (thousands)	Number	Funds (thousands)
Total	b/177	b/$10,438	b/177	b/$10,438
Center for Population Research	b/152	b/8,375	b/145	b/7,805
Extramural Programs:				
Perinatal Biology & Infant Mortality Branch	3	122	2	72
Growth & Development Branch	8	1,103	6	1,033
Adult Development & Aging Branch	7	329	5	263
Mental Retardation Branch	7	509	6	468
Epidemiology & Biometry Branch	-	-	6	520
Intramural Research Programs	-	-	5	189
Program Planning & Evaluation	-	-	2	89

a/ Includes one scientific evaluation grant for $125,000 not assigned to a program area.

b/ Includes 12 contracts totaling $989,000 funded through PASA agreement with the Agency for International Development.

Notes: (1) One scientific evaluation research grant for $160,000 is included in MR.

(2) Dollar figures may not add to totals due to rounding.

NICHD - PSAB
April 16, 1971

Number

Mechanism of Support	1964	1965	1966	1967	1968	1969	1970
Total Grants	1,013	1,222	1,444	1,531	1,393	1,331	1,176
Research	784	934	1,091	1,125	1,008	904	842
Training	74	92	132	146	150	151	134
Fellowships	88	119	141	166	135	173	86
RCP	67	77	80	94	100	103	114
Contracts	6	30	24	35	38	a/ 112	143

Funds (thousands)

Mechanism of Support	1964	1965	1966	1967	1968	1969	1970
Total Grants	$27,017	$34,586	$45,451	$49,790	$51,396	$51,800	$53,106
Research	21,612	26,524	34,303	36,787	37,985	37,596	40,099
Training	3,515	5,506	8,296	9,279	9,747	10,012	9,392
Fellowships	649	1,024	1,155	1,616	1,338	1,740	871
RCP	1,241	1,533	1,697	2,107	2,326	2,452	2,744
Contracts	138	1,264	946	2,520	2,150	a/ 6,310	7,678

a/ Includes 28 contracts totaling $1,496,000 funded through a PASA agreement with the Agency for International Development.

Note: Dollar figures may not add to totals due to rounding.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Scientific Conference Branch

The responsibilities of the Scientific Conference Branch include the duties of Council Secretary, Committee Management and the management of NICHD operated conferences.

The National Child Health and Human Development Council met in November, March and June, but regrettably without the full complement of members due to delays in the approval of Council members at the Departmental level.

The statement on Organization of DHEW Population and Family Planning Activities approved by the Secretary recommended that the National Advisory Child Health and Human Development Council be expanded from twelve to fourteen appointed members. Expansion of the Council to fourteen enables us to provide additional expertise for the expanded population program activity. Also because of the awareness and interest of young people the Department is encouraging the involvement of youth and students in Government and the decision making process by appointing young people to Public Advisory Committees. The NIH recommended that one young person be appointed to each Advisory Council. NICHD has such a person nominated to fill this category.

The Department has revised its system of processing persons for appointment to Public Advisory Committees. Previously a nomination was submitted and a pre-appointment name check of the individual was made. Currently, nominations are submitted and after approval is received the appointment of the nominee can be made immediately. After the appointment is complete a post-appointment name check is made by the Department.

The NICHD Committee Management Office has processed 24 appointments under the new guidelines for appointments during FY 71.

There are currently 8 NICHD Advisory Committees including the National Advisory Child Health and Human Development Council. A total of 90 members are serving on these committees. On February 11, 1971, the Population Research Advisory Committee was abolished.

In FY 71 this Branch assisted in the planning and arrangements for 21 conferences under the direct sponsorship of NICHD. Approximately 525 scientists participated in these meetings; almost half of them were first-time participants in Institute conferences.

The conferences by program area were:

<u>Office of Program Planning and Evaluation</u>

4th Conference on Behavioral Sciences and Medical Education

Child Development and the Man-Made Environment

Research Directions Toward Reduction of Injury in the Very Young and the Very Old

Center for Population Research

Research Approaches to Sex Education, Contraception, Family Planning and Morality

Consequences of Population Change in Developed Societies

Perinatal Biology and Infant Mortality Branch

Horizons in Perinatal Research

Designs for Special Care Facilities for Newborns

Animal Models in Perinatal Research

Sensory Needs of Hospitalized High Risk Newborn Infants

Growth and Development Branch

Assessment of Tests of Behavior in Studies of Nutrition in the Western Hemisphere

Communicating by Language: Relationships of Speech and Reading

Adult Development and Aging Branch

Regional Conference on the Biology of Aging

Intermediary Metabolism and Aging

Progeria and Aging

Development of an Information System on Animal Models for Research in Aging

2nd Regional Workshop on the Biology of Aging

Workshop on In Vitro Studies of Aging

Menopause and Aging

Consultation on Demographic Aspects of Aging

Mental Retardation Branch

Human Cytogenetics

Evaluation of Methodological Approaches to the Study of Brain Maturation and its Disturbances

Plans have been initiated for 18 conferences during FY 72.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Scientific Information Centers Branch

The objectives of the Scientific Information Centers Branch is to assist all researchers working in the broad fields of the Institute's program areas. The objectives of the Branch are five-fold: 1) to collect, evaluate and store all information in the Institute's areas of interest, 2) to keep researchers current with the published literature by publishing monthly abstracts journals for each program area, answering specific inquiries from scientists in the field and publishing bibliographies on very specific subjects, 3) to keep the researchers aware of the current research in their respective fields by means of current surveys which list the ongoing research grants and contracts, 4) to analyze and evaluate information so that evaluative reviews of specific areas of research can be published periodically and 5) to edit and publish scientific monographs and conference proceedings for the Institute.

The Branch is currently publishing experimental issues of two journals, "Adult Development and Aging Abstracts" and "Population and Reproduction Research Abstracts" and Population Research" for the fiscal year.

OFFICE OF THE BRANCH CHIEF

TECHNICAL PUBLICATIONS

The Technical Publications Section made a compilation and index of NICHD reprints covering the period 1969 and 1970. This will be a yearly undertaking of this unit.

The Section had edited and published the proceedings of a conference "Issues in Human Development" edited by Dr. Victor Vaughan. It also published "Sudden Infant Death Syndrome," a summarization for interested lay audience of progress in this area. Currently undergoing edit are two publications, "Malnutrition and Brain Function: Neurophysiological Methods of Assessment," Drs. Read, Prescott and Coursin, Scientific Editors and the Oogenesis Conference publication, Dr. John Biggers, Scientific Editor.

The Section is overseeing "Perspectives in Cytogenetics; the Next Decade," Dr. Stanley Wright, Scientific Editor, to be published by a Commercial Publishing House.

CURRENT RESEARCH SURVEYS

This Section has published the third annual surveys of research and research-related activities "Population Research: 1969." This compilation includes research projects, training grants and other related activities of governmental agencies (federal, state and local) and the not-for-profit organization (philanthropic foundations, voluntary health agencies and universities). The unit also prepared a report on "The Federal Program in Population Research." This is an inventory of the population research supported by federal agencies during fiscal year 1970. It updates and expands a previous report of projects active during fiscal year 1969.

DATA PROCESSING SECTION

The three IBM 2741 remote terminals which replaced the IBM 029 keypunch devices as the prime input mode to the computer provided the section with improved efficiency. The change-over ran smoothly on the part of the personnel involved. The Branch's input was exclusively through the DCRT Central Processing System and the WYLBUT package.

REFERENCES SERVICES

In November the NICHD library was moved from Building 31 to the Westwood Building and a library liaison office was established in Building 31. The space assigned the library in the Westwood Building was not large enough to accommodate the full collection of 4,593 books and 461 periodicals. In order to maintain the full collection, the professional staff selected 600 books and kept them in their respective offices. The librarian in Building 31 will recall these books and circulate to other staff members, upon request.

The library liaison office in Building 31 houses all the reference materials and the librarian fills all interlibrary loan requests and assists the staff in making searches.

The library statistics for 1970 are:

Books and Periodicals circulated	1,667
Interlibrary Loans filled	1,392
Requests items to be reproduced	554
Quick Reference Questions	531
Long Reference Questions	116
Books Purchased	1,302
Periodicals Purchased	18

LITERATURE PROCESSING SECTION

During the fiscal year, the staff continued processing literature for both the "Adult Development and Aging Abstracts" and "Population and Reproduction Research Abstracts." The staff evaluated 310,000 articles for pertinency to the above program areas.

The staff also made comprehensive literature searches for fourteen NICHD staff members and one search for Dr. Eisdorfer of Duke University and one for the Librarian of the University of Texas.

Staff Activities:

Mrs. Madge Goolsby, NICHD Librarian, was appointed Vice Chairman of the Montgomery County, Md., Library Board in 1970. She is also the Membership Chairman of the Biology Division of the Washington Chapter of the Special Libraries Association.

Mr. Martin Peller of the Current Survey Unit served as a member of the Board of Directors of the Association of Population Libraries and Information Centers. He participated in the organization of the National meeting of the Association to be held in May 1971 at the University of North Carolina.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971
Office of Associate Director for Intramural Research Programs

The programs of Intramural Research, NICHD have, during the past year, shown progress in a number of areas and contributed significantly to the acquisition of new knowledge in problems of development and aging. Reorganization of the Gerontology Research Center has placed the Branch Chiefs in a position to exercise direct responsibility for the program content in a way similar to that enjoyed by Branch Chiefs on the Bethesda campus. The reproduction research program has expanded markedly by the transfer of the Endocrinology Branch of NCI to NICHD. Dr. Mortimer Lipsett was made Associate Scientific Director for Reproduction while retaining his position of Chief of the Reproduction Research Branch.

In general, programs have shown considerable consolidation and branches, newly organized during the previous fiscal year, have begun to function effectively. A Pregnancy Research Branch was established and a Chief, Dr. Ronald Chez, recruited. Operation of this Branch will not begin until fiscal year 1972. A Clinical Director, Dr. John Robbins, joined the staff. He has begun intense research activity in the pathogenesis of infections in human infants, the development of the immune system and the natural history of infections with the bacteria H. Influenza. Within the next fiscal year his program will be incorporated into a Developmental Immunology Branch. Total budgeted positions in intramural research has increased from 224 to 244. In addition a total of 69 scientists are working in the Institute laboratories as guest workers, guest scientists, visiting fellows, visiting scientists, and visiting associates. While the dispersed nature of the activities of the Institute have presented many handicaps, it has been possible to slowly weld the branches into an organized unit with interplay and interaction among the component parts.

Office of Clinical Director

1. Microorganism possessing cross reacting antigens with the Hemophilus influenza organism have been identified. These antigens appear to be products of microbial metabolism rather than structural elements as in the case of the capsular polysaccharide of H. Influenza. It has been demonstrated in rat models that an antigen derived from E. coli can protect against lethel infections with H. Influenza. These experiments form the basis for proposing that much of the so called "natural immunity" to known pathogenic organisms in man and animals is derived not from infection but, rather, as a result of antibody production in response to antigens produced by unrelated organisms.

2. A lymphocyte surface structure apparently resulting in impairment of the ability to impede colonization by H. Influenza has been identified and found present in all subjects with severe infections due to this organism. The defect appears to be inherited as an autosomal dominant characteristic.

Gerontology Research Center - Accomplishments

During the year considerable progress has been made in developing new knowledge of understanding the basic mechanisms of aging. This includes data derived

from studies conducted at various levels: Molecular, cellular, organ and whole animal.

Clinical Physiology Branch

1. A non-invasive technique for the assessment of ventricular function by computation of systolic time intervals has been applied to 333 normal men aged 20-93. In subjects with no clinical evidence of heart disease, the total ejection time and the pre-ejection phase increase to a maximum at age 60. After age 60 both variables decline. Since these increasing values may reflect a decrement in ventricular function with age, the failure of average values to increase after age 60 may be a reflection of selective mortality, subjects with high values may have higher mortality rates in later life so that cross-sectional data may not show a further increase after age 60. The longitudinal follow-up of individual subjects will eventually answer this important question.

2. Using glucose $1^{14}C$ in tracer studies it was shown that with the infusion of insulin the decrease in hepatic glucose production and increase in peripheral glucose utilization was the same in old and young subjects. Furthermore, metabolic recycling of glucose did not differ with age. The conclusion that aging reduced β cell sensitivity is further strengthened.

Laboratory of Behavioral Sciences

1. It has been shown that problem solving ability declines significantly among men who were tested initially after the age of 60 and retested six to eight years later. This effect is not present among subjects who were under the age of 60 for their first test. The evidence indicates that the decline among the older men resulted from an inability to make logical inferences from already available information. This experimental measure of problem solving performance is probably assessing cognitive functions which are different from those measured psychometric tests since previous longitudinal studies which have used psychometric measures of cognition have reported no age-related changes.

2. Studies of the ability of patients with cardiac disease to control selected aspects of their cardiac function, and studies of the mechanisms by which monkeys control their heart rates are continuing. Results from experiments with five patients with atrial fibrillation indicate that these patients can consistently, differentially control their heart rates, i.e, the patients can voluntarily and reliably speed and slow their hearts. These data should help resolve a controversy which has been in the cardiological literature ever since Sir Thomas Lewis concluded that ventricular rate in atrial fibrillation is a random event. Apparently it is not. More importantly, however, these data suggest that it is possible to significantly modify some functions of the atrio-ventricular node through volitional action.

3. Ongoing experiments with monkeys are assessing the relation between state of arousal, and direction of control of pulse rate. Animals are trained to speed and slow heart rate. At the same time that the animal is learning heart rate control, or performing at a high level of learning, the percentage time during which it is producing high voltage, synchronized brain waves is also being recorded. Preliminary findings suggest that voluntary cardiac slowing is associated with a relatively "relaxed" brain while voluntary cardiac

speeding is associated with a more "aroused" brain.

Laboratory of Cellular and Comparative Physiology

1. Studies have shown that, with increasing age, cells in a variety of animals (rotifers, crayfish, mice, rats) show an increased accumulation of lysosomes and lysosomal enzymes which break down cytoplasmic substances. Similar increases in lysosomal enzymes have been found in cells or organs undergoing hormone induced involution. Thus further evidence is accumulating to indicate the importance of lysosomes in cell loss which is associated with aging.

2. The decrease in maximum O_2 uptake of mitochondria isolated from insect flight muscle observed in old flies is localized in the respiratory chain. Ultrastructural studies showed a unique degradation of mitochondrial cristae in mitochondria from old flies.

Laboratory of Molecular Aging

1. Polymers of nucleotides and their analogs have been designed, synthesized, and evaluated for their selective uptake into cells with the view of conferring viral protection and potentially of influencing the aging process. It has been found that changes in the potent interferon inducer, poly I poly C, that modify the polymer backbone substantially, such as loops on 2'-5' phosphodiester bonds, lead to decreased antiviral activity. However, the complex of polyinosinic acid and poly(1-vinylcytosine) shows high interferon induction activity. It is postulated that the high antiviral activity is related to the reduction of the charge/mass ratio and to the existence of this complex in an aggregated state. These factors generally enhance their transport into cells.

2. The healing surgical wound has been used as a model system to examine the mechanisms of aging of soft connective tissues. Young animals, rats of 8 months of age, produce a significantly greater mass of scar tissue in response to incision than do 18 month old rats and this is reflected in an increased breaking strength for the scar tissue of the younger animal. The tissue produced by the younger animal is more compliant (extension at break is greater) than is that produced by the older animals. This indicates the presence of cellular mechanisms in the older animals which induce its regenerated tissues to have one of the characteristics associated with aging in connective tissue, namely, increased stiffness.

3. In investigations of the mechanisms of the decreases in muscular work performance during aging using the flight of insects as the model system, it was found that isolated flight muscle mitochondria from senescent flies have a significantly lower State 3 rate of respiration (the maximal rate of O_2 uptake obtainable in the presence of excess substrate, ADP, and oxygen) than those from mature flies. Neither the "resting" State 4 rate nor the ADP/O ratio is perturbed by age. The decreased State 3 rate is obtained with the two physiological substrates of this muscle, α-glycerophosphate and pyruvate+proline, indicating that the decrement in oxidative capacity is localized in the respiratory chain. Correlated ultrastructural studies reveal an unique degradation in the mitochondrial cristae in old flies. In related studies

of the mechanisms of regulation at the critical enzymatic sites of carbohydrate utilization in this muscle, a novel control of phosphorylase b kinase by calcium ions and inorganic phosphate has been discovered and partially characterized. Activation of the kinase is essential for the initation of glycogenolysis. The enzyme trehalase, which is activated when the utilization of blood sugar is initiated, has been localized in the inner membrane of the mitochondrion. An isozyme is also found in the cytosol fraction of the muscle.

4. For the first time, the kinetics of binding of glucose to isolated renal brush borders have been examined as part of a study on the mechanisms of active transport. At least two types of binding sites have been identified that differ greatly in their affinity for glucose. The high affinity system is half-saturated at about 0.6 μM whereas the low affinity site is half-saturated at 25 mM. Binding is readily reversible. Phlorizin competitively inhibits the high affinity binding but inhibits the low affinity system non-competitively. These and other results are consistent with the concept that binding of glucose to the brush border membrane is an integral step in the transtubular transport of the sugar.

Collaborative Guest Scientist Program

During the past year the Collaborative Guest Scientist Program has been initiated. Eight guest scientist programs have been established in the Center. These programs are presently staffed by 19 scientists and 12 technicians and have underway 7 projects. Several important observations have been made by scientists working in this program.

1. The non-intestinal effects of cholera toxin administered intravenously to dogs, mice and rats have been studied. Biochemical determinations on serum and specific tissues (liver, blood platelets) add to the growing evidence that cholera toxin alters cell function by activating adenyl cyclase activity. The effect of the toxin is delayed in onset, prolonged duration, and active on a variety of cell types. This suggests that cholera toxin may prove a unique tool for the manipulation and study of adenyl cyclase-cAMP mediated processes. It may also be extremely useful for studying the functions of aging cells and organs.

2. Dogs immunized with cholera toxin plus adjuvant were protected against living vibrio challenge for over 10 months after immunization. This protection correlated with serum antitoxin titers. Jejunal response to the toxin was significantly reduced following passive transfer of antitoxic serum. Intestinal fluid from convalescent cholera and non-cholera patients contained considerable toxin-neutralizing capacity, not present in patients with the acute disease or in normal controls. This material neutralized both cholera toxin and E. coli enterotoxin. In at least half of the patients it retained full activity after 30 minutes boiling, suggesting it was not a protein antibody. It is known that acute infectious diarrheas decrease in incidence with increasing age but the mechanisms involved are not clear. Acquired antibacterial immunity may play a role or antitoxic immunity might occur. The demonstration of an acquired heat-stable antitoxic material suggests that non-immune mechanisms may also be involved. Further study of these mechanisms may help explain the means by which the small bowel is usually maintained

nearly bacteria-free.

3. The way in which purified cholera toxin interacts with cells is being investigated. Epididymal fat pads from rats are incubated in collagenase and passed through silk netting. The resulting isolated fat cell suspension is then incubated under various conditions to explore the nature of cell-toxin interactions. The cholera toxin stimulated the breakdown of triglyceride to glycerol and free fatty acids. This reaction does not depend on protein synthesis but on the effect of calcium, magnesium, and potassium ions. The lipolytic stimulating effect is locked with specific antisera permitting the system to be used to titrate both toxin and antitoxin. The age of rats used to obtain the lipocytes is important since the older rats showed higher threshold to the toxin effects. Adenyl cyclase was also stimulated and cyclic AMP levels raised by preincubating fat cells with toxin.

Laboratory of Biomedical Sciences - Accomplishments

Section on Molecular Genetics

1. Recent work in the Section has delineated genetically the mechanism involved in the insertion and excision of the phage λ into and from its host's genome. By removing the usual attachment site for this phage, it has also been possible to insert into many points the host genome and thus to produce a wide variety of phage, containing small portions of the host chromosome. These are vital to subsequent studies requiring a relatively purified set of genes and should prove extremely useful to the field in general.

2. Two cell-free protein synthesizing systems have been derived from bacteria and from Krebs II ascites cells. Both of these appear to translate phage and viral mRNA under conditions which give rise to authentic viral proteins. The bacterial system has been used to show that repression of a phage function by its own coat protein prevents translation of the first two codons of the repressed function. Thus the site of action of this repressor and the elucidation of a very narrow range of nucleotide sequences corresponding to the initial region of the repressed cistron has been determined.

Section on Developmental Enzymology

1. Five placental enzymes are under study: Glucose dehydrogenase has been purified 5000-fold and has been characterized with regard to pH optimum substrate affinities, and molecular weight; lactic acid dehydrogenase; glucose-6-phosphate dehydrogenase; 6-phospho gluconate dehydrogenase; beta glucuronidase and hexosaminidase. Methods have been devised for demonstrating isoenzyme patterns of the first three enzymes. During growth and development, the enzymes may show developmental variations in isoenzyme pattern, in total activity, or in both. Significant changes in levels of activities occur in half of the enzymes studied. In addition, striking changes in isoenzyme patterns and in total enzyme activities were observed in two placentas associated with "blighted" ovum abortions. This is the first study demonstrating qualitative and quantitative changes in enzyme activity in abnormal products of conception.

Section on Intermediary Metabolism

1. It has been demonstrated that p-chlorophenylalanine and phenylalanine treatment in the newborn rat produces a persistent neuropathology and an irreversible deficit in the adult rat's behavior in a number of test situations.

2. The first and second enzymes of pteridine biosynthesis in Comamonas sp. have been characterized and the first intermediate has been shown to be as a new compound, a cyclic phosphate of neopterin.

3. It has been shown that rat brain uses uridine in preference to orotic acid as a precursor of pyrimidines, a finding which indicates that de novo pyrimidine synthesis plays a relatively minor role in brain nucleic acid metabolism.

4. The complete purifications and characterization of a phenylalanine binding protein from Comamonas has been accomplished, and the amino group of lysine has been implicated as a component of the binding site.

Section of Physiological Controls

1. An extremely sensitive assay for cyclic AMP has been developed in order to study the effects of glucose-6-phosphate and glycogen in the regulation of glycogen synthetase phosphatase.

2. A cyclic AMP-dependent protein kinase has been obtained from rat liver, and has been partially purified and separated into subunits.

3. An isolated rat liver preparation, an analogue model to glucose (catabolite) repression in bacteria, has been developed and partially characterized with regard to the action of glucose on cyclic AMP production.

4. N-acetyltransferase has been characterized with regard to its pattern during development and its regulation by environmental lighting and neural pathways. Stimulation of the enzyme by norepinephrine has been shown to be mediated by a receptor which activates adenyl cyclase and increases cyclic AMP production. Substrate stabilization of the active form of the enzyme has been demonstrated.

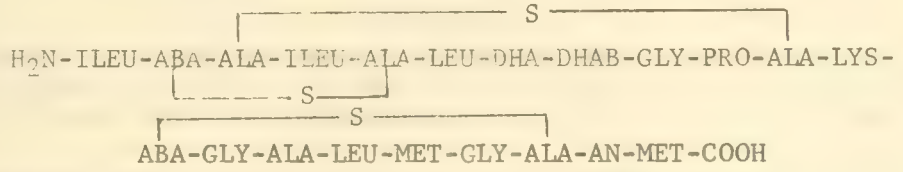
Section on Developmental Pharmacology

1. It has been possible to establish a chronological sequence of events occurring during aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons in cultures of fetal rat liver. These include: Passive intracellular entry and binding of inducer; synthesis of induction-specific RNA; translation of this RNA into induction-specific protein(s); appearance of a high-spin CO-binding cytochrome associated with hydroxylase activity, and enzymic metabolism of the inducer leading to covalent binding of some metabolites to cellular material and predominantly to excretion of metabolites from the cell. With cultures from certain mouse strains or from rat hepatoma in which hydroxylase induction does not occur, evidence has been obtained that the defect is transcriptional. In mouse genetic studies the response of hydroxylase induction

in liver, bowel, or kidney to systematic polycyclic hydrocarbons follows a Mendelian autosomal dominant pattern.

Section on Molecular Structure

1. The primary structure of nisin has been completely elucidated with the exception of the assignment of two sulfide bridges and the clarification of a molecular rearrangement. The structure of the H_2N-terminal fragment of nisin is as follows:



The structural elucidation of subtilin has been facilitated with the successful fragmentation of the molecule by trypsin and thermolysin under specifically established conditions. This partial structure may presently be written for subtilin: H_2-N-TRY-LY-LAN, B-CH_3-LAN_2, GLU_2, PRO, GLY_2, VAL, LEU_2, ALA-PHE, ASP, B-CH_3-LAN_2-GLU, LEU_2-LYS-ILEU-DHA-LYS-COOH. The presence of two residues of dehydroalanine and one residue of B-methyldehydroalanine is now firmly established.

2. Studies underway indicate that the lysosomal membrane contains a protein (or proteins) of relatively low molecular weight, approximately 6000.

3. Intraperitoneally administered nisin causes fetal resorption in the rat to the extent of 90%. The peptide appears to display this biological activity throughout the entire gestational period, the phenomenon more appropriately to be described as "inhibited implantation" for the early days of rat pregnancy. Intraperitoneally administered nisin also induces fetal resorption in the rabbit. The peptide shows little or no effect on the rat conceptus when given orally.

Children's Diagnostic and Study Branch - Accomplishments

1. Study of perceptual functioning in a group of children identified as having reading disability were screened to eliminate those with discernable neurological problems. After screening it was found that only one of the 54 was a female. All subjects showed superior ability to perform visual ordering of material and information. The studies suggest that there is a hereditary component transmitted by an x-link recessive gene which confers upon males this ordering superiority but in addition confers inefficiency in reading. This is presumably a result of inefficient facility for three dimensional coding and processing. This compromises their ability to see the letters b-p-q-d. It is possible that English orthography is particularly difficult for these males, for in Japan where different script is used, the incidence of reading disability is less than 1% whereas in American school children (mainly among males) it may be as high as 5%.

2. Studies in a patient with the Prader-Willi Syndrome in which there is

anatomic evidence of loss of hypothalamic tissue it was possible to demonstrate a change in the circadian periodicities of a variety of biologic mordalities. The studies in this subject subsequently extended through collaboration to include two other similarly affected patients, strongly indicate that the hypothalamic region controls circadian rhythm in human subjects.

Behavioral Biology Branch - Accomplishments

Section on Brain and Behavior

1. The EEG of naturally deprived rhesus monkeys show abnormal patterns particularly in the slow wave stage of sleep indicating a diffuse cerebral dysfunction rather than abnormalities in the specific sensory or motor systems. No abnormalities in blood platelet serotonin levels have been found.

2. Studies on processing in the central auditory system of the squirrel monkey have revealed that the response of a given neuronal unit may be due to two or three key features of a given call and that other features of the call make relatively little difference to the cell's responsiveness. Thus, in the primary auditory cortex, single neurons seem capable of signalling the occurrence of certain key features of vocalizations in these animals.

Section on Neurobiology

1. Cell proteins of a single snail neuron has been analyzed for the molecular weight distribution. Cell #11 in the nervous system has been chosen because of very large changes in its activity during the course of hibernation and activation. On the basis of the large granules revealed by electron microscopy, this cell can be classified as a neurosecretary cell. A prominent peak at a molecular weight of about 5000 daltons is observed when the cell is taken from an active animal but this peak is completely absent in the hibernating animal. This peak is unique to this cell among those studied. Thus, this cell has a characteristic electrophysiologic pattern which has now been described in detail, has a characteristic electron microscopic appearance and a cell specific protein which is qualitatively changed as a function of the state of the animal.

2. Two clones of neuroblastoma cells have been examined in detail with regard to electrical excitability and have been found to differ very markedly from one another. One line of cells has a relatively weak electrical activity even under conditions of maximum differentiation of the line. Another line of cells has a larger percentage of cells which stop dividing and differentiate, both morphologically and electrophysiologically. Neuroblastoma cells have been found to respond to application of acetylcholine with inhibitory or excitatory changes in membrane potential. Atropine and curare can partially or completely block these responses. The neuroblastoma thus provides a good model system for the study of cell process formation, electrical excitability and chemosensitivity.

3. The electrophysiology of nerve cells from chick spinal cord has been studied as they develop for dissociated cultures and establish functional, synaptically linked nets of neurons. They establish functional neuromuscular

contacts with muscle cells from embryonic chick. Excitatory and inhibitory synaptic potentials occur in the neurons and cholinergic synapses are formed between the nerve and muscle cells with most of the characteristics of normal neuromuscular synapses.

Reproduction Research Branch - Accomplishments

1. A fundamental question about gonadotropic hormones has been resolved by use of tissue culture of human trophoblast cells. It has been shown that gonadotropin heterogeneity is due, in large part, to the synthesis of gonadotropins with different sialic acid content.

2. It has been possible to form viable hybrids of human trophoblasts with specific lines of mouse and human cells. Since the trophoblasts have four metabolic markers and several distinctive marker chromosomes, the system will be exploited for studying gene function in mammalian cells.

3. It has been shown that the sialic acid moiety of the glycoprotein hormones, the gonadogropins, is the major determinant of metabolic half life as well as biological potency.

4. The binding of gonadotropins to receptors in the ovary and testis has been a focus of interest. The binding of FSH to the estrogen-stimulated ovary of the rat has been described. Luteinizing hormone and HGG have been shown to be bound with high affinity using intact and broken cell preparations from rat testis. In this system, binding depends on the specific polypeptide chain with no contribution from the carbohydrate fraction. It has also been possible to follow testosterone production by the Leydig cells in vitro in the same system.

5. For the first time evidence has been obtained that, in man, activity of the germinal epithelium of the testis modulates the release of FSH, a most important finding for studies of control of spermatogenesis.

Social and Behavioral Sciences Branch - Accomplishments

1. In a study of environment of six month old infants from lower socioeconomic backgrounds, it was found that it may not be very meaningful to label environments grossly as depriving. Relatively low relationships between dimensions of the inanimate and social environments have been found and dimensions of the early environment are significantly related to specific aspects of infant functioning. Level of social stimulation relates significantly to measures of the infant's social responsiveness and early vocalization; properties of inanimate stimulation, particularly variety, are more strongly related to the Bayley Psychomotor Development Index than to measures of exploratory behavior and preference for novel stimuli. Measures of cognitive-motivational functions, which index the infant's persistence and active attempts to reach out and have an effect on the environment, are significantly related to both characteristics of the inanimate and the social environment.

2. In a study of the effects of experiences in a Headstart program on the personality and cognitive development of children from "disadvantaged" backgrounds, it was found that there were few significant relationships between

cognitive input and changes in cognitive functions. Significant relationships were found between the teachers effective interactions with the children, the extent to which she identified and labelled feelings, and changes in the children towards a more positive self-concept.

3. In adopted children a relation between mothering experiences in infancy and cognitive and personality development at ten years has been observed. Several dimensions of maternal care were significantly related to IQ and to the child's capacity to establish discriminating relationships at ten years of age. These relationships are significant only for boys. An environment in infancy characterized by warmth, responsiveness to the child's cues, and one in which stimulation is sensitively adapted to the child's developmental level, is favorable for cognitive and emotional development of boys.

Developmental Biology Branch - Accomplishments

1. It has been observed that the lens from the eye of a six-day old chick, when excised and grown in tissue culture dramatically reduces the complexity of the protein profiles that are synthesized; within three days only a single species, that with a molecular weight of 45,000 is produced. The events in tissue culture closely mimic those found in normal embryonic development. This particular model can be exploited for the understanding of the differentiation which occurs when a single protein is made. Eventually, this single protein becomes lens crystalline.

2. Studies of microtubular formation in six-day old chick embryo lens preparations have provided information on cell differentiation. Microtubules form as these cells elongate, a process prerequisite for lens fiber formation. Disruption of microtubules by colchicine or vinblastine inhibit fiber cell formation but not protein synthesis. On the other hand, inhibition of protein synthesis by cycloheximide neither prevents fiber cell formation nor interfers with formation and orientation of microtubules. These results strongly suggest that ultimate cell structure relates to microtubule formation and conformation and apparently at least in part is independent of protein synthesis. These studies provide important insight into the biochemical control of differentiation.

3. Studies of formation of lens fiber proteins in excised six-day old chick lenses have shown that inhibition of RNA synthesis with actinomycin inhibits synthesis of proteins with molecular weights between 65,000 and 95,000 but not those with molecular weights of 45,000. Experiments with actinomycin and cycloheximide have demonstrated that the mRNA coding for epithelial proteins with molecular weights between 65,000 and 91,000 is unstable but those coding for proteins with molecular weight of 45,000 are stable. These studies suggest possible roles for stability of mRNA in controlling differentiation since the 45,000 molecular weight protein is the principal one in lens crystalline and becomes virtually the only protein synthesized by lens cells as they elongate and become lens fibers.

4. The presence of RNA-instructed nucleic acid polymerse activity has been demonstrated for the first time in fertilized sea urchin embryos. Activity has been systematically characterized with regard to dependence on exogenous

templates, enzyme concentrations, cation requirements, temperature sensitivities, and kinetics. This form of activity has to-date only been demonstrated in relation to RNA oncogenic viruses, certain non-oncogenic RNA viruses and two presumably virus-free mammalian cell lines cultured in vitro. The present findings, therefore, represent the first documentation of the presence of "reverse transcriptase" activities in embryos. The observations are important not only for their novelty, but because they offer a possible explanation for a means of intercellular communication via RNA and a scheme for incorporation of new information into the genome. It also offers a means for intercellular amplification of appropriate RNA transcripts acting in conjunction with RNA-instructed DNA polymerases. This mechanism may provide a means whereby DNA may differ among different cell-types within the organism and therefore permit cells to have a divergence of genomic information.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Office of Clinical Director

The research activities of the Office of the Clinical Director are concerned with two scientific problems. The first, is the disease caused by Hemophilus influenzae type b in humans. Several research programs have been designed to study the mechanisms of the pathogenesis, host susceptibility (acquired and constitutive), and the prevention of this disease. These programs have as their specific aims the isolation and characterization of the purified type b capsular polysaccharide (thought to be the protective antigen), and its production on an industrial scale, identification of non-pathogenic organisms with cross-reacting antigens which has been postulated by this group as the etiology of most adult "natural" immunity, creation of a laboratory model using experimental animals that will permit the study of Hemophilus influenzae type b disease, further identification of the lymphocyte surface structure present in most of individuals and one of their parents who have suffered from Hemophilus influenzae type b diseases (constitutive factor), and the immunogenicity and protective effect induced by immunization of infants and children at Charlotte, North Carolina with the purified type b capsular polysaccharide.

The second research problem is biologic role of serum alpha-1 anti trypsin inhibitor. This serum protein is present in high concentration of normal humans. The genetic control of its structure, its function in vivo and its precise relation to the development of the pulmonary disease in young aduls with a genetically directed deficiency of this serum protein are the problems under consideration. To study this problem, a method of isolation of mouse serum alpha-1 trypsin inhibitor was devised utilizing a novel adaptation of theory of polyacrylamide gel electrophoresis. The material has been used to induce mono-specific antiserum. Molecular weight determinations, available for the human protein has permitted an estimate of carbohydrate determinations and antigenic structure to be made. Fluorochrome derivatives have been prepared using anti alpha-1 trypsin inhibitor antibodies to study the cellular basis of this protein's biosynthesis.

Serial No. CL-1(c)
1. Office of Clinical Director
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Identification and characterization of cross reacting antigens to the type b capsular polysaccharide of H. influenzae from mixed bacterial cultures.

Previous Serial Number: None

Principle Investigators: Major W. Bradshaw, M. D.
Rachel Schneerson, M. D.

Other Investigators: John B. Robbins, M. D.

Man Years:

Total: 12/12
Professional: 12/12
Others: None

Project Description:

Objectives - To identify the chemical structure, cellular localization, immunogenicity in purified form and using whole acetone killed and living bacteria in animals, and occurrence in nature of bacterial antigens cross reactive with the type b capsular polysaccharide of Hemophilus influenzae.

Methods Employed - Several experimental observations had directed our efforts to test the possibility that the "natural" immunity, observed in most adults and presumed to be due to serum anti capsular antibody, must be stimulated by bacteria other than Hemophilus influenzae. To test this hypothesis, a technique was used to identify bacteria with cross reacting antigens from mixed cultures taken from the gastrointestinal and pharyngeal flora of normal rabbits and humans. In this study, anti type b polysaccharide serum, prepared in goats and sheep by intravenous injection of whole encapsulated Hemophilus influenzae organisms, was incorporated into agar plates. Hemophilus influenzae and Diplococcus pneumoniae type 29 (an organism previously shown to contain a capsular polysaccharide cross reactive with the type b substance) yielded halos of precipitation surrounding the individual bacterial colonies, presumably due to the release of antigenically precipitable material into the antiserum agar. With this technique, an Escherichiae coli from a normal individual (designated strain Easter), several Staphylococcus aureus and S. epidermidis and several Streptococcus viridins non-groupable were shown to yield a specific halo. To extend this study, the Center for Disease Control, Atlanta, Georgia; Dr. Marvin Turck, USPHS Hospital, Seattle, Washington; and Dr. Fritz Orskov, WHO Escherichiae Center, Copenhagen, Denmark were

contacted and a search for organisms with a similar structure is under investigation. To date, three additional E. coli isolated from the collection at the Center for Disease Control have been identified. Two organisms have had the serologic identity of O:75,H:5 and O:5,H:5. The material isolated from these E. coli has been shown to be capsular in cellular localization by immunofluorescence and to be a polysaccharide containing ribose by direct analysis of the sugar. Isolation of the cross reacting material from the streptococcus viridans has been accomplished but the high degree of purity achieved for the E. coli material has not yet been recognized. This material is antigenically identical to the type b capsular polysaccharide of Hemophilus influenzae. Immunization of sheep and goats with whole encapsulated E. coli has produced antiserum shown to be protective in mouse lethal infection assays (using mucin enhancement) and in the complement dependent bactericidal assays with virilent strains of H. influenzae. Absorbtion of anti H. influenzae type b serum and anti E. coli serum with the E. coli antigen has been shown to remove protective activity from both reagents. Although studies are incomplete, preliminary evidence indicates that the antigen isolated from the three E. coli is protective, antigenically identical to the polysaccharide of H. influenzae and occupies the same cellular localization. Its preliminary characterization would seem to put it in the classification of "K" antigen, usually acidic polysaccharides which are capsular. Using the 90 antisera present in the WHO Escherichiae Center, no specificity of K has been attached to this material as of yet.

Honors and Awards: None

Publications: None

Serial No. CL-2
1. Office of Clinical Director
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 throught June 30, 1971

Project Title: Purification and physicochemical characterization and biological properties of mouse serum alpha-1 antitrypsin

Previous Serial Number: None

Principle Investigator: Richard L. Myerowitz, M. D.

Other Investigators: John B. Robbins, M. D.
Andreas Chrambach, M. D.
David Rodbard, M. D.

Cooperating Units: Reproduction Research Branch, NICHD

Man Years:

Total: 12/12
Professional: 12/12
Others: None

Project Description:

Objectives - A disease state has been described in humans characterized by a genetically controlled deficiency of a serum protein (alpha-1 antitrypsin) and a progressive, fatal, youthful emphysema unrelated to environmental exposure. The pathogenesis of this pulmonary lesion is unknown but the direct relation between the homozygous serum protein deficiency and the pulmonary affliction is well documented. The human serum protein exhibited micro-heterogeneity with an antigenically identical "fast" and "slow" variant in normals. The homozygous deficient state is characterized by an absolute deficiency of the "fast" variant.

The objectives of this study are to isolate the serum alpha-1 trypsin inhibitor in a laboratory animal (mouse), to identify the microheterogeneity and define any genetic polymorphism, to study by physiologic and biochemical techniques the possible physiologic function of this protein to identify the biologic function, and to study the cellular basis of its synthesis (cellular localization and biosynthetic control).

Serum alpha-1 antitrypsin has physical and chemical properties very similar to serum albumin. In man, purification of the functionally active serum protein has been difficult. In the mouse preliminary observations revealed that the electrophoretic mobility of the alpha-1 antitrypsin with respect to

serum albumin was dissimilar in two types of supporting gels. In polyacrylamide gels of high concentration it is observed that the electrophoretic mobility of this protein increased relative to albumin indicating that there was a molecular size and charge difference between alpha-1 antitrypsin and its nearest neighbor and usual contaminant, serum albumin. Accordingly, electrophoretic mobility of serum albumin and serum alpha-1 antitrypsin were determined in polyacrylamide gels of varying concentration and pH. By extrapolating these values, it was found that conditions could be achieved for preparative polyacrylamide gel electrophoresis for the separation of this alpha-1 antitrypsin from all other serum proteins. Preliminary to the preparative polyacrylamide gel electrophoresis procedure, serum albumin was removed from fractions separated by DEAE cellulose chromatography by passage through an insoluble anti-mouse column (affinity chromatography). The resultant preparation was found to be essentially pure and free of contamination by any other serum protein. The specific antitryptic activity, molecular weight, and carbohydrate content is now under investigation.

The biologic properties of this serum protein can now be investigated through studies of the action and metabolism of the purified protein in vivo, as well as by immunofluorescent studies of mouse tissues in vitro using antisera raised against the purified protein.

To date, a molecular weight determination of 35,000 was done by the sedimentation equilibrium method. Antigenic analysis of this purified protein has revealed microheterogeneity with a "fast" and "slow" component, similar to those observed in humans. Unlike the heterologous antiserum, human alpha-1 antiserum system, antigenic differences are demonstrable between these variants. This antigenic difference, revealed by the antiserum, will be used to determine the cellular basis of the synthesis of these two variants.

Honors and Awards: None

Publications: None

Serial No. CL-3(c)
1. Office of Clinical Director
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Adaptation of the Colorimetric Assay of Serum Trypsin Inhibitory Capacity to the Auto-Analyzer

Previous Serial Number: None

Principle Investigator: Richard L. Myerowitz, M. D.

Other Investigators: John B. Robbins, M. D.
Irving Eckman, M. S.

Cooperating Units: State University of New York, Downstate Medical Center, Brooklyn, New York

Man Years:

Total: 36/12
Professional: 36/12
Other: None

Project Description:

The measurement of serum trypsin inhibitory capacity (90% of which is due to serum alpha-1 antitrypsin) by the colorimetric method of Eriksson using the chromagenic ester substrate BAPNA, although tedious, represents the most available screening procedure for the detection of patients with elevated levels of serum alpha-1 antitrypsin as well as those with genetic deficiency of this protein with its associated pulmonary emphysema. This method, without essential modification, has been adapted to the Auto-Analyzer.

A linear relationship between the percent inhibition of tryptic activity, as determined by the Auto-Analyzer, and increasing concentrations of pure soybean trypsin inhibitor has been demonstrated. It has been shown that human and mouse sera with different levels of serum trypsin inhibitory capacity demonstrated levels of percent inhibition that correlated well with a simultaneous immunochemical determination of alpha-1 antitrypsin by the Auto-Analyzer using monospecific antisera. Human serum, however, appears to contain chromagens which are detected by the colorimeter and, therefore, falsely reduce the apparent percent inhibition. Studies are in progress to determine if the magnitude of this "quenching" effect is similar for most sera. If so, the addition of this value to the observed percent inhibition could allow for accurate determiantion of serum trypsin inhibitory capacity by

this rapid automated technique. Even if accurate determination is not possible, this technique would still be useful for automated screening for alpha-1 antitypsin deficiency.

Honors and Awards: None

Publications: None

Serial No. CL-4
1. Office of Clinical Director
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Age Related Susceptibility to Hemophilus Influenzae Type b Infections in Rabbits

Previous Serial Number: None

Principle Investigator: Rachel Schneerson, M. D.

Other Investigators: John B. Robbins, M. D.

Cooperating Units: None

Man Years:

Total: 12/12
Professional: 12/12
Other: None

Project Description:

Objectives - To determine if the inverse relation between the presence of serum capsular antibodies and susceptibility to influenzae meningitis shown to occur in humans can be demonstrated also for laboratory animals. It has been stated that H. influenzae type b is not a true pathogen for laboratory animals based upon many observations which indicated that large numbers of H. influenzae type b organisms injected with non-specific irritants are capable of inducing H. influenzae type b meningitis in laboratory animals.

Method Employed - Serum anti capsular antibodies were measured in animal sera by use of a passive hemagglutination test employing gluteraldehyde fixed erythrocytes and the purified type b capsular polysaccharide. The organism employed to test the susceptibility of these rabbits to infection was H. influenzae type b, strain Rab, utilized for the purification of the type b polysaccharide.

The anti type b antibody content of the sera of rabbits of varying ages was measured by studying animals from three different sources. All groups of animals were either taken from outdoor hutcheries of commercial breeders or the NIH hutchery.

Major Findings - The presence of anti type b antibodies in rabbits was similar to that observed in humans. Newborn and adult animals (greater than 6 weeks of age) had anti type b antibodies in their sera (approximately 95%). Animals from 1-3 weeks of age had no detectable type b antibody.

Examination of the nasopharynx and rectum of animals of varying ages showed a variety of bacteria with cross reacting antigens to the type b capsular polysaccharide, but in no case was H. influenzae type b isolated. We concluded that the antigenic stimulus for the synthesis of anti type b antibodies is probably other than H. influenzae type b.

Animals with pre-existing antibodies of about 1-3 weeks of age were highly susceptible to fatal septicemia with H. influenzae type b. Approximately 50% of animals without pre-existing anti type b antibodies died within a variable period of time (1 day - 2 weeks) following intravenous injection of 1×10^3 organisms. At levels of 10^5 organisms, the mortality was greater than 90%. Of interest was the observation that H. influenzae type b could be cultured in the blood for two weeks without any detectable change in the animals' general health and well being. Meningitis or pneumonitis was detected in many animals. Animals with pre-existing anitbody whether maternally acquired after birth or naturally acquired during adult life or administered passively were highly resistant to levels of organisms which ranged from 10^5 to 10^7. At doses of 10^8 and 10^9 organisms all animals died soon after IV injection (less than 2-3 hours) due to toxic effects.

It was concluded that an experimental model for septicemia and meningitis with H. influenzae type b could be established in laboratory animals. The failure of previous investigators to establish a laboratory model for H. influenzae type b disease could be attributed to the presence of serum type b antibodies in rabbit sera following 8 weeks of age. The source of antigenic stimuli to produce anti type b antibodies is probably due to cross reacting antigens present on other bacteria than H. influenzae type b.

Honors and Awards: None

Publications: None

Serial No. CL-5(c)
1. Office of Clinical Director
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Characterization of the Type b Polysaccharide Extracted from Hemophilus Influenzae Suitable for Mass Prophylaxis

Previous Serial Number: None

Principle Investigator: John K. Whisnant, M. D.

Other Investigators: John B. Robbins, M. D.

Man Years:

Total: 24/12
Professional: 24/12
Other: None

Project Description:

Objectives - The type b polysaccharide of Hemophilus influenzae has been shown to be immunogenic in humans. The protective quality of this immunization will be analyzed in field trial to consist of 3,000 immunized infants and children at the Charlotte Memorial Hospital. To produce sufficient type b capsular polysaccharide of Hemophilus influenzae for immunization, methods have been developed for mass production utilizing 10, 50 and 300 liter fermentors in a sequential fashion. The methods for isolation and characterization of this type b polysaccharide and comparison with material produced on a small scale previously shown to be immunogenic was the subject of this research project.

Methods Employed - Levinthal base, a necessary ingredient for the growth of H. influenzae type b, is a viscous material. Sufficient quantities for this project were prepared by sequential hemolysis of whole horse blood, high speed preparative ultracentrifugation, and filtration through Millipore membranes. A strain of H. influenzae type b shown to be stable under conditions of culture (not to revert to rough, unencapsulated variety) was used. This strain, designated Rab, was passed through three series of fermentations within 24 hours. Following the last growth which achieved a density of 6.0×10^9 organisms/ml and a final yield of 1.8×10^{15} organisms was isolated from whole fluid by high speed ultracentrifugation. The polysaccharide was eluted from the bacteria by mechanical agitation by formaldehyde buffered saline at room temperature and the purified material treated with 20% and then 70% ethyl alcohol first to remove the nucleic acids and then to precipitate the polysaccharide from solution. The polysaccharide was purified from the solution by extraction with aqueous phenol (45%) and shown to contain little

or no detectable nitrogen by chemical analysis. The molecular weight of the material , an important determinant for its immunogenicity, was determined by gel filtration in a dissociating solvent (8M urea) and these results compared to molecular weight determinations achieved by high speed ultracentrifugation, sedimentation velocity toward equilibrium and by viscosity analysis. It was shown that by adjusting and maintaining buffer values to avoid the unusual viscosity effects observed with polyanions and dilute aqueous solutions that this simple measurement (viscosity) could be used to determine with a fair degree of accuracy, the molecular weight of the purified polysaccharide. The material thus prepared was shown to have the characteristics of the type b polysaccharide purified in small amounts and shown to be immunogenic in humans.

Honors and Awards: None

Publications: None

Serial No. CL-6(c)
1. Office of Clinical Director
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Identification of a lymphocyte surface structure related to susceptibility to disease caused by Hemophilus influenzae type b

Previous Serial Number: None

Principle Investigator: John K. Whisnant, M. D.

Other Investigators: Dean Mann, M. D.
Nicholas Rogentine, M. D.
John B. Robbins, M. D.

Man Years:

Total: 12/12
Professional: 12/12
Other: None

Project Description:

This project is designed to identify a structure present in lymphoid cells that may explain the predisposition of some individuals to serious disease with Hemophilus influenzae type b. A search for a relationship between a cell structure and susceptibility to an infectious disease has been suggested by previous work indicating that the immune response to simple determinants may be under genetic regulation and that there is a defined relationship between some bacterial surface structures and human transplantation antigens.

To identify a cell surface structure two indicator reagents are being used. The first are the serologic reagents used to identify the HLA transplantation histocompatibility antigens on peripheral blood lymphocytes. The second is a highly purified goat antibody to the type b capsular polysaccahride of H. influenzae. The interaction between these sets of reagents and human lymphocytes is measured by the in vitro cytotoxicity test of Terasaki. Individuals who have had serious disease with H. influenzae type b, such as epiglottitis and meningitis, and their siblings and parents are used as subjects. Peripheral blood is drawn from these individuals and the reaction of these two sets of reagents is measured.

The genetics of susceptibility to Hemophilus disease was investigated also with the known segragating antigenic markers on red blood cells. All patients and family members were phenotyped in order to find some marker

linked to the lymphocyte susceptibility being sought.

This experimental approach has been utilized to study nine families of individuals suffering from severe H. influenzae type b disease. The experimental results to date show no definite histocompatibility phenotype preponderence in individuals with H. influenzae type b disease, their siblings or parents. The distribution of HLA antigens in these subjects is, with one possible exception, the same as the rest of the population. A unique relationship between the presence of a lymphocyte reactive substance and the anti type b sera has been found in patients with H. influenzae type b disease. The susceptibility of lymphocytes to anti type b serum is present in 1-3% of families in the general population but has been detected in lymphocytes from individuals of four of the nine families studied. The 60% of individuals or their families with H. influenzae type b disease show a cell surface structure reactive with the anti type b sera in contrast to the level of 1-3% in the general population. Red cell phenotyping has thus far not produced a marker linked to this interesting phenomenon. However, this part of the study has shown two very rare red cell antigens in only the eight families completed. In addition, two individuals with disease failed to show one antigen which they should have inherited.

These results to date will be extended to the study of approximately twenty families. If these unusual relationships persists, it can be stated that a genetically determined structure has been found to coincide with susceptibility to Hemophilus influenzae type b disease. It will be the subject of future research to decide the nature of this reactive structure and the alteration of host defense that permits susceptibility to this disease.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1970 through June 30, 1971

Office of Clinical Director
Contract and Collaborative Research

Contract Title: Develop and Test Capsular Polysaccharide of Hemophilus Influenzae Type b

Contractor: Charlotte Memorial Hospital
P. O. Box 2554
Charlotte, North Carolina

Money Allocated: $32,755.00

Objectives: To confirm the immunogenecity of the type b polysaccharide in infants and children. Following this, the protective effect against H. influenzae type b diseases of immunization will be assayed in Charlotte, N. C.

Methods: Diseases caused by H. influenzae type b constitute a serious cause of morbidity and mortality in the United States. Meningitis, the most common disease produced by the organism, occurs most often in infancy and childhood. Approximately 500 deaths due to this disease are reported annually. Further, there are about 1500 deaths reported as due to unspecified bacteria meninigitides, and it is likely that many of these are due to H. influenzae type b. These are minimum estimates of the mortality due to H. influenzae type b meningitis since culture and identification of this organism is not possible in many hospitals and meningitis due to this organism, not considered to be contagious, is not always reported. Only an estimate may be made of the morbidity of this disease because the incidence of H. influenzae type b meningitis is unknown. The incidence of H. influenzae type b meningitis may be inferred from reports that indicate that approximately 5-10% of infants and children affected succumb to this disease. Based upon these statistics, we have inferred that there are approximately 10,000 to 20,000 cases of H. influenzae type b meningitis annually. Although effective chemotherapy is available for this disease, various workers have reported that 25-50% of individuals "recovering" from H. influenzae type b meningitis have residual central nervous system deficits including deafness, behavioral disturbances and mental retardation.

The threat to the health of our citizens posed by disease due to H. influenzae type b may be compared to that due to polio and measles virus and meningococcal meningitis. In 1951 (prior to immunization) there were 3,100 deaths from polio virus and in 1960 there were 600 deaths from measles and 950 deaths from meningococcus.

There is no available data to estimate the mortality and morbidity of other diseases caused by H. influenzae type b. Epiglottitis, arthritis, osteomyelitis, pneumonitis and cellulitis are not reportable diseases and the mortality to these diseases is not listed in the Statistis of the NCDC or the U. S. Bureau of Vital Statistics. However, epiglottitis, an acute upper

respiratory infection produced by H. influenzae type b has a high mortality and morbidity.

Mechanisms of Immunity - Serum antibody (maternally derived and "naturally" acquired during development) has been presumed to be protective. The specificity of these protective antibodies has been presumed to be the capsular polysaccharide. This concept is based upon the reported loss of activity of hyperimmune serum (prepared by injection of whole organisms) following absorption by the purified type b substance. The origin of the antigenic stimulus that induces "natural" immunity is unknown. Anti capsular antibodies may be synthesized following disease caused by H. influenzae type b but the low nasopharyngeal carriage rate, and infrequency of disease with this organism support the concept that Hemophilus influenzae type b is not the only source of "natural" immunity. Some cross reactions have been reported between the type specific substance of pneumococci and our laboratory has demonstrated that many alpha-streptococci and some E. coli possess an antigenically similar polysaccharide.

The protocol is designed to test the efficacy and safety of injecting the capsular polysaccharide during infancy and childhood. The first experiment will be designed to ask the following questions: "What is the optimal dose and preparation of polysaccharide injected into 2 month old infants for induction of serum antibody formation?" Four groups of five infants each aged 6 weeks to 2 months will be studied. The first and second groups will receive 1 and 5 mcg. respectively of the capsular polysaccharide suspended in physiologic saline subcutaneously coincident with their DPT immunization. The second and third groups will receive 1 and 5 mcg. respectively of the capsular polysaccharide suspended in aluminum hydroxide subcutaneously. All infants will be bled prior to injection and at their second DPT (age 3 months) and third DPT (age 4 months). Serum antibodies to the capsular polysaccharide and diptheria antigen will be assessed by hemagglutination and a radioactive antigen assay. To evaluate whether the capsular polysaccharide will induce a depressant effect upon the immune response to other antigens, anti-diptheria antibodies will be assayed in the laboratory of Dr. John B. Robbins, NICHD, by the intracutaneous test of Fraser. In addition, nasopharyngeal cultures of these infants and controls will be done to identify any effect upon the nasopharyngeal carrier state of the organism.

Honors and Awards: None

Publications: None

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971
Developmental Biology Branch

The Developmental Biology Branch continues under its present structure with an Acting Branch Chief. The programs of the laboratory are concerned largely with early molecular events associated with fertilization and biochemical changes and control mechanisms for differentiation. The principal models have been the chick embryo and the sea urchin egg.

It has been observed that the lens from the eye of a six-day old chick, when excised and grown in tissue culture dramatically reduces the complexity of the protein profiles that are synthesized; within three days only a single species, that with a molecular weight of 45,000 is produced. The events in tissue culture closely mimic those found in normal embryonic development this particular model can be exploited for the understanding of the differentiation which occurs when a single protein is made. Eventually, this single protein becomes lens crystalline.

Studies of microtubular formation in six-day old chick embryo lens preparations provides information on cell differentiation. Microtubules form as these cells elongate, a process prerequisite for lens fiber formation. Disruption of microtubules by colchicine or vinblastine inhibits fiber cells formation but not protein synthesis. On the other hand, inhibition of protein synthesis by cycloheximide neither prevented fiber cell formation nor interfered with formation and orientation of microtubules. These results strongly suggest that ultimate cell structure relates to microtubule formation and conformation and apparently at least in part is independent of protein synthesis. These studies provide important insight into the biochemical control of differentiation.

Studies in this same preparation were conducted to determine the relation between lens fiber formation and synthesis of RNA. It was then demonstrated that the initiation of lens fiber formation is associated with a marked increase of all types of RNA and especially of rRNA. The study demonstrates that the elongation of cultured lens epithelial cells involves a stimulation having a genetic basis that prints genes to a different degree during specific phases of differentiation.

Studies of RNA synthesis during oogenesis indicate that the principal RNA form is ribosomal and the synthesis begins very early in oogenesis. Of special interest have been observations made on mitochondria during oogenesis. It is not possible to demonstrate any specific proteins synthesized from ribosomes of mitochondrial RNA. Most, or possibly all, of the protein synthesis occurring after artificial activation of non-nucleate fragments of sea urchin eggs is synthesized upon RNA templates which were present in unfertilized eggs. Despite this apparent limited information role, synthesis of mitochondrial RNA has been demonstrated in the presence of ethidium bromide and thus mitochondrial genetic material (DNA) may be important for expression of normal morphology.

Studies of formation of lens fiber proteins in excised six-day old chick lenses has been conducted. Inhibition of RNA synthesis with actinomycin inhibits synthesis of proteins with molecular weights between 65,000 and 95,000 but not those with molecular weights of 45,000. Experiments with actinomycin and cycloheximide have demonstrated that the mRNA coding for epithelial proteins with molecular weights between 65,000 and 91,000 is unstable but those coding for proteins with molecular weight of 45,000 is stable. These studies suggest possible roles for stability of mRNA in controlling differentiation since the 45,000 molecular weight protein is the principal one in lens crystalline and becomes virtually the only protein synthesized by lens cells as they elongate and become lens fibers.

The presence of RNA-instructed nucleic acid polymerse activity has been demonstrated for the first time in fertilized sea urchin embryos. Activity has been systematically characterized with regard to dependence on exogenous templates, enzyme concentrations, cation requirements, temperature sensitivities, and kinetics. This form of activity has to-date only been demonstrated in relation to RNA oncogenic viruses, certain non-oncogenic RNA viruses and two persumably virus-free mammalian cell lines cultured in vitro. The present findings, therefore, represent the first documentation of the presence of "reverse transcriptase" activities in embryos.

The observations are important not only for their novelty, but because they offer a possible explanation for a means of intercellular communication via RNA and a scheme for incorporation of new information into the geno. It also offers a means for intercellular amplification of appropriate RNA transcripts acting in conjunction with RNA-instructed DNA polymerases. This mechanism may provide a means whereby DNA may differ among different cell-types within the organism and therefore permit cells to have a divergence of genomic information.

Studies of RNA synthesis and concomitant protein synthesis in early embryogenesis have been conducted. It was found that although synthesis of ribosomal RNA and ribosomal protein may be initiated early in embryogenesis, these new molecules apparently are not utilized in the assemblage of functional ribosomes. This redundnacy has been provisionally termed transcriptive wastage. The purpose of this function, remains to be defined.

Serial No. HD-DB10
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Synthesis of Proteins During Lens Fiber Cell Formation In Vitro.

Previous Serial Number: HD-DB10

Principal Investigator: Joram P. Piatigorsky, Ph.D.

Other Investigators: Sydney P. Craig, III, Ph.D.
Henry deF. Webster, M.D., NINDS

Cooperating Units: Developmental Biology Branch, NICHD

Man Years

Total:	0.3
Professional:	0.3
Other:	0

Project Description

Objectives: It has been shown that excised lens epithelial cells of 6 day chick embryos elongate in culture to form prospective lens fibers. The purpose of this project is to characterize the proteins made by lens epithelial cells as they elongate in culture to form fiber cells.

Methods Employed: Epithelial cells of 6 day chick embryo lens were excised and explanted into serum-containing culture. They were removed at various times, up to 3 days, and labelled with radioactive amino acids. The labelled proteins were extracted and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The appearance of new proteins were followed by double label experiments in which the proteins synthesized by the epithelial cells were compared directly with the proteins synthesized by the elongating fiber cells. Similar double label experiments were performed with the epithelium and fibers of the intact lens.

Major Findings: Excised lens epithelia synthesize proteins ranging from a molecular weight of at least 3×10^5 to 2×10^4. The major components have molecular weights of 91,000, 65,000, 45,000, 27,000 and 20,000. Each labelled polypeptide of a given molecular weight probably consists of several different proteins. Experiments are in progress to fractionate these.

After 24 hours in culture, the epithelial cells no longer synthesize appreciable amounts of the proteins with a molecular weight of 91,000 or 65,000. Those with molecular weights of 45,000, 27,000 and 20,000 continue to be synthesized.

This pattern of synthesis continues for at least 3 days in vitro. The number of newly synthesized proteins is reduced even further in the fibers of the intact lens. These synthesize predominantly the proteins with a molecular weight of 45,000. The cells located in the equatorial region of the intact lens epithelium synthesize the same proteins as those in the central epithelium, except that the proteins with a molecular weight of 45,000 are labelled approximately 30% more than those in the central cells relative to the other newly synthesized proteins. Equatorial epithelial cells are beginning to elongate in the 6 day lens.

The formation of lens fibers, then, is associated with a reduction in the number of new proteins. Those with a molecular weight of 45,000 predominate. The changes in protein synthesis during lens fiber formation in relatively short-term cultures approximate those that take place in vivo.

Significance to Biomedical Research and the Program of the Institute: The association of specific proteins with specialized cells is considered to be an important criterion of cell differentiation. The study of the lens fiber cell formation has the specific advantages that it can be followed in vitro under relatively simple conditions, and that there are already a few specific proteins, the lens crystallins, which are known to be associated only with lens cells. These studies increase our knowledge of the regulatory mechanisms involved in the appearance of specific proteins during the differentiation of lens fiber cells.

Proposed Course of Project: The results of this project are presently being prepared for publication. Further investigations are planned for the characterization of the proteins synthesized during lens fiber formation and for study of the mechanism by which such synthesis is regulated.

Honors and Awards: None

Publications: J. Piatigorsky, H. deF. Webster and S. Craig: Ultrastructural and biochemical aspects of lens development in vivo and in vitro. J. Cell Biol. 47: 158a - 159a (1970).

Serial No. HD-DB10(1)
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Characterization of Newly Synthesized Proteins and Differential Stabilities of Messenger RNA's in the Cultured Lens Epithelium of the Chick Embryo.

Previous Serial Number: None (further extension of HD-DB10)

Principal Investigator: Sydney P. Craig, III, Ph.D.

Other Investigators: Joram P. Piatigorsky, Ph.D.

Cooperating Units: Developmental Biology Branch, NICHD

Man Years

Total:	0.3
Professional:	0.3
Other:	0

Objectives: Cells in the cultured epithelium of the 6 day chick lens elongate into fibers if serum is added to the medium. Many more proteins are synthesized by the epithelial cells then by the fibers in the intact lens. The simplified pattern of protein synthesis characteristic of lens fibers accompanies the elongation of the cultured epithelial cells. The purpose of this project is to characterize the proteins synthesized by the epithelial cells in the absence of serum (non-elongating conditions) and to investigate the possibility that different proteins are synthesized upon messenger RNA's of different stability. This would suggest that the formation of lens fibers is associated with a loss of specific messenger RNA's and a retention of others which were synthesized in the epithelium.

Methods Employed: The epithelia of 6 day embryonic chick lenses were excised and cultured in Ham's F-10 medium lacking valine. The epithelia were labelled with radioactive valine for various lengths of time in the presence or absence of dactinomycin. In some experiments the epithelia were pre-labelled with radioactive valine and chased with dactinomycin and/or cycloheximide. The labelled proteins were extracted and examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis and by isofocusing in sucrose density gradients.

Major Findings: The major peaks of proteins synthesized by cultured epithelia have molecular weights of 91,000, 65,000, 45,000, 27,000 and 20,000 when analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The principal proteins, consisting of some 40% of all the labelled proteins, are those with a molecular weight of 45,000. It is this species which is predominantly syn-

thesized by lens fibers. These proteins consist of at least 2 polypeptides, a major species with an isoelectric point of 5.12 and a minor species with an isoelectric point of 6.17. The isoelectric points of the proteins with other molecular weights are under investigation.

Inhibition of RNA synthesis with dactinomycin severely inhibits the synthesis of proteins with molecular weights of 65,000 and of 91,000, while the synthesis of proteins with a molecular weight of 45,000 is unaffected. The effect of dactinomycin on protein synthesis in the excised epithelia closely resembles the change in the pattern of protein synthesis which occurs during the formation of lens fibers by the epithelial cells cultured with serum. Pulse-chase experiments with dactinomycin and cycloheximide indicates that the inhibition of synthesis of the proteins with a molecular weight of 91,000 and 65,000 is not caused by a high rate of degradation of these proteins either in the presence or in the absence of dactinomycin. Rather, it appears that the messenger RNA's which direct the synthesis of proteins with a molecular weight of 65,000 and 91,000 in the epithelium are unstable, while those acting as template for the proteins with a molecular weight of 45,000 are stable.

Significance to Biomedical Research and the Program of the Institute: Very little is known about the regulation of specific proteins during development and differentiation of animal cells. The cultured lens epithelium provides a model system in which differentiation appears to proceed *in vitro* similarly to that *in vivo*. One of the important criterion for differentiation is the change in synthesis of specific proteins. These studies increase our knowledge of the mechanisms involved in the change in the pattern of protein synthesis during the specialization of embryonic eukaryotic cells.

Proposed Course of Project: The physical properties of the proteins synthesized by the cultured lens epithelium will be characterized further. Since differential stabilities of messenger RNA's have been implicated as an important mechanism of regulating protein synthesis during the formation of lens fibers, this aspect of the project will include a study of RNA synthesis as well as protein synthesis.

Honors and Awards: None

Publications: None

Serial No. HD-DB8
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electron Microscopic Study of the Formation of Microtubules During Lens Fiber Cell Formation In Vitro and In Vivo.

Previous Serial Number: HD-DB8

Principal Investigator: Joram P. Piatigorsky, Ph.D.

Other Investigators: Henry deForest Webster, M.D., NINDS
Maureen O'Connell, NINDS
Sydney P. Craig, III, Ph.D.

Cooperating Units: Developmental Biology Branch, NICHD

Man Years

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description

Objectives: It has been reported previously that microtubules form along the periphery of the cell during the elongation of epithelial cells into fiber cells in the embryonic chick lens. The purpose of the present study is to confirm the formation of microtubules in the intact lens and to determine whether or not microtubules are also associated with the formation of fiber cells from excised lens epithelial cells in tissue culture.

Methods Employed: Intact lens of 6 day and 12 day chick embryos are removed and fixed with 2% glutaraldehyde and 2% osmium, embedded in araldite, thin sectioned and examined with the electron microscope. Epithelia from 6 day chick lenses are excised and cultured with serum for 1 to 6 days and examined electron microscopically at various time intervals. Colchicine and vinblastine sulfate, both agents which disrupt microtubules, are used in culture to test the importance of microtubules for the elongation of the epithelial cells in vitro as normally occurs with serum.

Major Findings: Cultured epithelial cells elongate to form fiber cells. This elongation has been found to be linear for at least 5 days with a doubling of cell length having occurred after 24 hours. Microtubules have been observed in the electron microscope in epithelial cells as well as in fiber cells of the 6 day and the 12 day intact lens. In the fiber cells, however, they are more pronounced and more peripherally located than in the epithelial cells. Cultured epithelial cells which are elongating in culture also contain micro-

tubules. These appear to increase in number and become more aligned along the cell membrane during elongation in vitro. Disruption of the microtubules with colchicine or vinblastine sulfate inhibits fiber cell formation in culture. Neither of these agents affects protein synthesis. Inhibition of protein synthesis with cycloheximide, however, does not prevent the initial phases of fiber cell formation, nor does it prevent the formation and alignment of microtubules. Microtubular assembly thus is an important aspect of lens fiber formation.

Significance to Biomedical Research and the Program of the Institute: This investigation hopes to relate the shape of a specialized cell to an internal "skeleton", the microtubules, and to follow the development of the microtubules in culture. Since microtubular protein can be relatively easily isolated and studied, the visual presence of microtubules will be able to be related to either the synthesis or assembly of microtubular protein. Thus, one aspect of the normal differentiation of the lens fibers, namely the development of shape, will be characterized eventually at both the ultrastructural and the biochemical level. It is anticipated that this will be of importance to the understanding of the causative factors of cell shape as well as to the understanding of the normal sequence of events in the differentiation of lens fiber cells.

Proposed Course of Project: The electron microscopy will be terminated once the manuscript of this project is prepared. The synthesis of microtubular protein will be quantitated and studied further.

Honors and Awards: None

Publications: J. Piatigorsky, H. deF. Webster and S. Craig: Ultrastructural and biochemical aspects of lens development in vivo and in vitro. J. Cell Biol. 47: 158a - 159a (1970).

Serial No: HD-DB2

1. Developmental Biology Branch
2.
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effect of Serum on the Synthesis of RNA and of DNA in the Cultured Lens Epithelium of the Chick Embryo: Initiation of Lens Fiber Formation in vitro.

Previous Serial Number: None (extension of HD-DB2)

Principal Investigator: Joram P. Piatigorsky, Ph.D.

Other Investigators: Sonia S. Rothschild, M.A.

Cooperating Units: Developmental Biology Branch, NICHD

Man Years

Total:	0.75
Professional:	0.25
Other:	0.50

Project Description

Objectives: Excised lens epithelial cells of 6 day chick embryo elongate to form fiber cells in vitro if, and only if, serum is added to the medium. The purpose of this study was to determine whether or not gene activation is associated with the initiation of fiber formation in culture.

Methods Employed: Excised lens epithelia from 6 day chick embryos were labelled in vitro with either H^3-adenosine or H^3-methyl-methionine in the presence or absence of serum. The specific activity of the H^3-ATP pool was determined by use of the luciferin-luciferase reaction in combination with thin-layer chromatography. The kinetics of labelled S-adenosylmethionine formation was assayed by chromatography on cellulose plates. Labelled RNA was phenol extracted and examined electrophoretically on polyacrylamide gels. Incorporation of H^3-adenosine into DNA in the presence and absence of serum was also investigated. The labelled DNA was isolated by cesium chloride buoyant density gradient centrifugation. All radioactive assays were made by scintillation counting.

Major Findings: Serum stimulated the incorporation of H^3-adenosine into polydisperse and 4 S RNA's by about 2-fold and into 28 S and 18 S ribosomal RNA's (rRNA) by approximately 4-fold in 3 hours. These values were obtained after adjustment for the specific activity of the intracellular pool of H^3-ATP. Experiments with Actinomycin D showed that the greater incorporation was not due to slower degradation of RNA.

After 3 hours in the presence of H^3-methyl-methionine, serum increased the labelling of rRNA by about 3.8-fold. Serum stimulated the rate of methylation of 45 S precursor rRNA and the accumulation of methylated 32 S, 28 S and 18 S RNA's, while affecting neither the extent nor the rate of labelling of the S-adenosylmethionine pool.

Serum did not stimulate the rate of DNA synthesis in 3 hours, but possibly decreased it.

These results show that the initiation of lens fiber formation in vitro by serum is associated with an increase in the synthesis of all types of RNA, especially of rRNA.

Significance to Biomedical Research and the Program of the Institute: Differentiation of cells during embryogenesis is largely dependent on differential gene activation. The present study demonstrates that the elongation of cultured lens epithelial cells involves a stimulation of genetic activity. Different genes are stimulated to different extents. The cultured lens epithelium thus provides an ideal system in which to study the mechanism and role of gene activity during normal differentiation and morphogenesis.

Proposed Course of Project: The types of RNA's synthesized during lens fiber formation will be studied further by RNA-DNA hybridization. The regulation of RNA synthesis will be correlated with the changing pattern of protein synthesis during fiber cell formation both in vivo and in vitro.

Honors and Awards: None

Publications: J. Piatigorsky and S.S. Rothschild: Effect of serum on the synthesis of RNA and of DNA in the cultured lens epithelium of the chick embryo: Initiation of lens fiber formation in vitro. Biochim. Biophys. Acta 230: 86-98 (1971).

Serial No. HD-DB17
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Fractionation of Serum into Components Capable of Supporting Division or Differentiation of Lens Epithelial Cells.

Previous Serial Number: None

Principal Investigator: Joram P. Piatigorsky, Ph.D.

Other Investigators: Janice Blustein

Cooperating Units: Developmental Biology Branch, NICHD

Man Years

Total:	0.2
Professional:	0.05
Other:	0.15

Project Description

Objectives: Excised lens epithelial cells from the 6 day chick embryo elongate into lens fibers when the culture medium contains serum, while those from the 19 day embryo divide under the same conditions. Furthermore, serum stimulates mitosis in the 6 day lens epithelial cells if the explants have been deprived of serum for 8 hours or longer. The purpose of the present study is to fractionate serum in order to identify the component or components which support differentiation and to compare these with the serum components which stimulate mitosis.

Methods Employed: Lens epithelia of 6 day or of 19 day embryos are explanted into petri dishes and cultured in the presence of different serum components. After 3 days of culture the explants are examined histologically in paraffin sections. In order to assay for division, the cultured explants are labelled with H^3-thymidine and examined either by autoradiography or by scintillation counting. Serum is fractioned by dialysis in conventional tubing, by Sephadex chromatography and by ion-exchange chromatography.

Major Findings: This project is in its initial stages and only preliminary results have been obtained. Dialyzable materials in the serum do not appear to be capable of supporting differentiation of the 6 day lens epithelial cells. Serum macromolecules, however, appear to be capable of stimulating elongation. These are being fractionated further.

Significance to Biomedical Research and the Program of the Institute: Serum is a requirement for growth and differentiation of cultured cells from higher organisms. Neither the components nor their mechanism of action are known. The differing response of lens epithelial cells from early and late embryos to serum provides a system suitable to test the components of serum and separate those important for differentiation from those necessary for cell division. These studies will be of importance for establishment of cell cultures and for greater understanding of the mechanisms of mitosis and of differentiation of higher cells.

Proposed Course of Project: This project will be continued until serum has been fractionated into components necessary for division and for differentiation of cultured lens epithelial cells. The active components will then be characterized further.

Honors and Awards: None

Publications: None

Serial No. HD-DB18
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Lens Fiber Formation and DNA Synthesis in the Cultured Lens Epithelium of Chick Embryos at Different Stages of Development.

Previous Serial Number: None

Principal Investigator: Joram P. Piatigorsky, Ph.D.

Other Investigators: Sonia S. Rothschild, M.A.

Cooperating Units: Developmental Biology Branch, NICHD

Man Years

Total:	0.75
Professional:	0.25
Other:	0.50

Project Description

Objectives: Excised lens epithelial cells from 6 day chick embryos elongate in serum-containing medium and, according to autoradiographic data, stop synthesizing DNA. The purpose of the present study is to quantitate more accurately the rates of DNA synthesis before and during elongation of the cultured epithelia. The response of cultured lens epithelia from progressively older chick embryos was also investigated in order to determine (a) whether the ability to elongate is lost during development and (b) whether DNA synthesis would also decline in cultured epithelial cells which do not elongate in vitro.

Methods Employed: Lens epithelia of 6, 9, 12, 15 and 19 day chick embryos were cultured for 3 days in serum-containing medium. Separate cultures were examined histologically before and after incubation. For studies on DNA synthesis, explants were labelled with either H^3-adenosine or with H^3-thymidine. Some cultures labelled with thymidine were examined by autoradiography. In others, the DNA was isolated, purified by buoyant density gradient centrifugation and the new DNA quantitated by scintillation counting of the trichloroacetic acid precipitated fractions of the gradient. Explants labelled with H^3-adenosine were dissolved in 0.3N potassium hydroxide, incubated at 37° C for 18 hours to hydrolyze the RNA, and the DNA purified by Sephadex chromatography and quantitated by scintillation counting of the acid precipitable radioactivity. The values were adjusted on the basis of the endogenous specific activity of the H^3-ATP pool which was measured separately by use of the luciferin-luciferase reaction and thin-layer chromatography of the H^3-ATP.

Major Findings: Cultured lens epithelial cells from chick embryos up to 15 days of development elongate in serum-containing medium. The extent of elongation diminishes with age such that 6 day epithelial cells, on the average, approximately triple in length in 3 days while 15 day epithelial cells approximately double in length. Cultured 19 day epithelial cells, however, do not elongate *in vitro*.

Initially, DNA synthesis is about 10 times more active in 6 day lens epithelia than in 19 day epithelia. After 3 days of culture, the 6 day epithelia have decreased their rate of DNA synthesis by 3-fold, while the 19 day epithelia have increased their rate of DNA synthesis by 3-fold. Thus, cultivation of the lens epithelium from chick embryos causes elongation and decrease of DNA synthesis in 6 day explants, but initiates mitosis in 19 day explants.

Significance to Biomedical Research and the Program of the Institute: It has already been demonstrated in this laboratory that the elongating, cultured 6 day lens epithelial cells change in ultrastructural appearance and in pattern of protein synthesis in a direction similar to that which occurs during lens fiber formation *in vivo*. The demonstration that the elongating epithelial cells decrease their rate of DNA synthesis in culture provides another parameter which shows that the elongating cells behave similarly *in vitro* and *in vivo*. Furthermore, the fact that the ability of cultured lens epithelial cells is progressively lost during development also indicates that the *in vitro* elongation represents specific fiber differentiation, rather than non-specific growth. The cultured embryonic lens epithelium, then, provides a simple model system in which to study the molecular aspects of normal development.

Proposed Course of Project: This project is presently being prepared for presentation and will be terminated once the manuscript has been accepted for publication.

Honors and Awards: None

Publications: None

Serial No. HD-DB9
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mitochondrial RNA and Protein Synthesis: Studies on Oogenesis, Non-nucleate Egg Fragments and Embryos of the Sea Urchin.

Previous Serial Number: None (extension of HD-DB9 and HD-DB11)

Principal Investigator: Sydney P. Craig, III, Ph.D.

Other Investigators: Joram P. Piatigorsky, Ph.D.

Cooperating Units: Developmental Biology Branch, NICHD

Man Years

Total:	0.75
Professional:	0.75
Other:	0

Project Description

Objectives: The mitochondria of sea urchin eggs have been shown to contain DNA which is transcribed during early development. The purpose of this project is to determine the types of RNA's present in, and synthesized by, mitochondria during oogenesis and after fertilization, and also to explore what role these RNA's have in protein synthesis in the mitochondria and, possibly, in the cytoplasm.

Methods Employed: RNA's were labelled during oogenesis by injecting H^3-uridine into shed sea urchins and allowing the growing oocytes to incorporate the label. Labelled unfertilized eggs were obtained in intervals ranging from 3 days to 3 months after the injection of radioactivity. Embryos and activated non-nucleate fragments of eggs were labelled by incubation with H^3- uridine or with H^3- or C^{14}-valine. Labelled RNA's were extracted with phenol and analyzed by polyacrylamide gel electrophoresis, benzoylated DEAE-cellulose chromatography, DNA-RNA hybridization and sucrose gradient centrifugation. Labelled proteins were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Non-nucleate fragments were made by buoyant density centrifugation in sucrose-sea water gradients. Mitochondria were isolated from homogenates by differential and sucrose density gradient centrifugations.

Major Findings: (a). RNA synthesis during oogenesis: Ribosomal RNA is the main species of RNA synthesized during oogenesis and present in the unfertilized egg. This synthesis begins in very small oocytes and continues until the end of oogenesis. The mitochondria in unfertilized eggs are shown to be associated

with RNA's with a molecular weight of about 700,000 and of 360,000. These may correspond to mitochondrial ribosomal RNA as described in other systems. Mitochondria also contain significant amounts of low molecular RNA's which may be transfer RNA's. (b). Mitochondrial RNA synthesis during development: Labeling for short and long periods with H^3-uridine have indicated that mitochondria synthesize a relatively unstable population of polydisperse RNA's and a stable population of discrete RNA's with molecular weights of approximately 700,000 and 360,000 (possibly mitochondrial ribosomal RNA), and of about 25,000 (possibly transfer RNA's). These are under further investigation. (c). Mitochondrial protein synthesis during development: Proteins synthesis was explored in artificially activated non-nucleate fragments in the presence and absence of ethidium bromide which inhibits mitochondrial RNA synthesis. The electrophoretic pattern of proteins labeled in the activated fragments, in sodium dodecyl sulfate polyacrylamide gels, is not qualitatively altered by 6 hours of preincubation in ethidium bromide. The range of molecular weights for the labeled proteins is 9,000 to 40,000 for those found in the 15,000 x g supernatant fraction of the homogenate and 6,000 to 60,000 for those in the corresponding pellet (largely mitochondria). Similar results are obtained if mitochondria are purified from whole embryos by sucrose density gradient centrifugation. Thus, most, or possibly all, of the types of proteins labeled after artificial activation of non-nucleate fragments are synthesized upon RNA templates which are present in unfertilized eggs. So far, then, no specific protein has been detected which is known to be synthesized by mitochondrial RNA. (d). Possible role of mitochondrial genetic activity in morphogenesis: Developing embryos of sea urchins were exposed to various concentrations of ethidium bromide in order to test the morphological effect of suppressing mitochondrial RNA synthesis. Two micrograms per ml produces a prism stage larva with an abbreviated gut and 5 µg/ml arrests development at the blastula stage. At 10 µg/ml, development does not proceed beyond the 4- to 8-cell stage. When mesenchyme blastulae or gastrulae are treated with 10 µg/ml of ethidium bromide, development ceases but the embryos continue to swim for at least 30 hours. Two, 5 and 10 µg/ml of ethidium bromide inhibit mitochondrial RNA synthesis by about 50%, 80% and 97%, respectively. These results suggest that mitochondrial genetic activity may be important for normal morphogenesis. However, side effects of the ethidium bromide cannot be excluded at the present time.

Significance to Biomedical Research and the Program of the Institute: The discovery of DNA in mitochondria has suggested that mitochondria may have larger regulatory functions than previously realized. Sea urchin eggs are especially suitable for the study of mitochondria, since they have such a large cytoplasm to nucleus ratio thus having an excess of mitochondrial DNA. Furthermore, non-nucleate fragments can easily be made and mitochondrial genetic activity can be studied without nuclear interference. Finally, the role of mitochondria in development may also be investigated. These studies, then, are germane to the understanding of mitochondrial genetic activity during normal development.

Proposed Course of Project: The project will be continued with emphasis on characterization of the types of RNA's and of proteins made by mitochondria during development.

Honors and Awards: None

Publications:

1. Piatigorsky, J. and Tyler, A.: Changes upon fertilization in the distribution of RNA-containign particles in sea urchin eggs. Develop. Biol. 21: 13-28 (1970).

2. Craig, S. P.: Synthesis of RNA in non-nucleate fragments of sea urchin eggs. J. Mol. Biol. 47: 615-618 (1970).

3. Craig, S. P. and Piatigorsky, J.: Protein synthesis and development in the absence of cytoplasmic RNA synthesis in non-nucleate egg fragments and embryos of sea urchins: Effect of ethidium bromide. Develop. Biol. 24: 214-232 (1971).

Serial No. HD-DB13
1. Developmental Biology Branch
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ribosomal Diversification During Early Development
I. The Non-coordinate Synthesis of Ribosomal RNA and Ribosomal Protein
II. Recombination Between Maternal and Embryonic Subunits
III. Ribosomal-mediated Recognition of Specific Genetic Messages

Previous Serial Number: None

Principal Investigator: D. W. Slater

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives: To rigorously define the role of the ribosome in the selective expression of latent programs for protein synthesis which occurs during early embryogenesis and to experimentally evaluate the proposition that ribosomes are not monolithic.

Methods Employed: Sea urchin eggs and embryos are currently being utilized. To date, ribosomes, polysomes, their constituent macromolecules and various other cell fractions have been prepared from unfertilized eggs and metabolically diverse developmental stages employing both labeled amino acids and/or the relevant RNA precursors under a wide variety of experimental conditions, each designed to yield information prerequisite to the solution of one facet of the problem under investigation. Analysis of these cellular entities is proceeding through the use of the appropriate purification procedures and subsequent characterization on sucrose gradients, polyacrylamide gels, etc.

Major Findings: Experimental design necessitates that the spectrum of experiments undertaken be analyzed in tandem. Definitive statements regarding the major objectives of this project must therefore be deferred until all the necessary information has been obtained. Preliminary evidence, however, tentatively suggests that although the synthesis of ribosomal RNAs and

ribosomal proteins may be initiated earlier in embryogenesis than previously surmised, the former are apparently not utilized in the assemblage of functional ribosomes. This phenomenon has been provisionally termed "transcriptive leakage". Its implications may not be restricted to the synthesis of ribosomal RNA.

Significance to Biomedical Research and the Program of the Institute: In metazoans the transcription of a genetic message and its ultimate utilization as a template for protein synthesis are chronologically separable events definable in terms of hours or even weeks. This period of post-transcriptive latency is forcefully illustrated in the developing embryo, as early embryogenesis is contingent upon the ordered expression of maternal messages synthesized months prior to their utilization. Given the substantial population of such genetic templates present in the unfertilized egg, the resulting embryo must obviously possess suitable mechanisms for governing when in the course of development these specific programs for protein synthesis are initiated. Circumstantial evidence culled from eukaryote and prokaryote organisms now suggests that the ribosome itself is the depository of such information. Documentation of this proposition and the elucidation of the processes involved are therefore of immediate importance to our understanding of mammalian development and, further, appear to be a practical necessity if early embryogenesis is to be subjected to purposeful manipulation. Although the import of this research for the molecular basis of embryogenesis has been stressed, the potential implications with regard to the coordinate expression of non-linked genes as well as its relevance to genetic dominance are equally evident.

Proposed Course of Project: This project was not undertaken for the sole purpose of elucidating the molecular mechanisms governing sea urchin development. Rather, the choice of this material was partially dictated by logistical necessities. A sea urchin can yield over 2×10^6 normal eggs as opposed to the approximately 100 eggs obtainable from a superovulated mouse. Further, early development in both forms appears to be sufficiently similar to permit conditional extrapolation from the echinoid embryo to that of the mammal. Once the present series of investigations have provided the prerequisite information, however, it will be possible to assess the veracity of these findings in mammalian embryos with the maximal assurance of obtaining concrete results with the minimal expenditure of mammalian embryonic material.

Honors and Awards: None

Publications:

1. Slater, D. W., and Spiegelman, S.: Transcriptive Expression During Sea Urchin Embryogenesis. Biochim. Biophys. Acta. 213: 194-207, 1970.

Serial No. HD-DB14

1. Developmental Biology Branch
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: RNA-instructed Nucleic Acid Polymerase Activity During Early Embryogenesis

Previous Serial Number: None

Principal Investigator: D. W. Slater

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives: To determine whether RNA-instructed nucleic acid polymerases are present in normal eggs and embryos and to evaluate their role in early embryogenesis.

Methods Employed: To date, sea urchin eggs and embryos have been employed as experimental material. Suitable procedures have been developed for the preparation and assay of the pertinent polymerase activities as well as the resulting products.

Major Findings: Echinoid embryos contain a spectrum of potential nucleic acid polymerases. In addition to the expected DNA-dependent nucleic acid polymerases, we have now demonstrated the presence of RNA-instructed polymerase activities. These activities have been systematically characterized with regard to their dependence on exogenous templates, enzyme concentrations, cation requirements, temperature sensitivities, kinetics, etc. Manuscripts detailing one such activity, an RNA-directed DNA polymerase, are being completed for publication.

Significance to Biomedical Research and the Program of the Institute: Prior to the results reported herein, the demonstration of RNA-instructed DNA polymerase activity had been restricted to RNA oncogenic viruses, certain non-oncogenic RNA viruses and two presumably virus-free mammalian cell lines cultured *in vitro*. Our findings therefore represent the first documentation of the presence of "reverse transcriptase" activity in embryos actively

undergoing differentiation per se and, more important, possessing the potential for normal development.

In the interest of brevity, the obvious implications of this work for the origin of the polymerases associated with oncogenic viruses, antibody formation and transverse evolution will not be discussed. Rather, only those considerations as are of immediate import to development will be summarily mentioned.

The existence of mechanisms for transmitting genetic information from DNA through an RNA intermediate and subsequently into DNA provides a potential means for intercellular communication via RNA and the incorporation of this information into the genome. Here, the relevance of RNA-instructed DNA polymerases to primary embryonic induction must be considered. Further, since it is known that particular genes can be selectively amplified (as witnessed by the selective amplification of DNA cistrons coding for ribosomal RNA which occurs during oogenesis), such amplification may proceed through the use of appropriate RNA transcripts in conjunction with RNA-instructed DNA polymerases. Finally, our findings pose the question as to the degree of informational divergence in DNAs of different cell types within an organism and challenge the precept that all cells in a particular organism are necessarily endowed with equivalent genomes.

Proposed Course of Project: Investigations regarding RNA-instructed nucleic acid polymerase activities during sea urchin development will be expanded. Preparations are in progress to initiate similar studies in mammalian embryos.

Honors and Awards: None

Publications: None

Serial No. HD-DB - 5
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immune mechanisms in chronic and congenital viral infections

Previous Serial Number: Same

Principle Investigator: Lon R. White, M.D., DB, NICHD

Other Investigators: George Nemo, Ph.D.
Jacob A. Brody, M.D.
Harrie Anne Sutton, biologist, DB, NICHD

Cooperating Units: Epidemiology Branch, C & FR, NINDS
(corresponding project serial no. NDS(CF)-70 E 1833)

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: 1. To establish one or more models of chronic and/or congenital viral infection in experimental animals. 2. To define the development of cellular and humoral immunity to the same and to heterologous antigens.

Methods Employed: Neonatal and pregnant mice are injected with either a reovirus type 1 or the minute virus of mice (MVM). The persistence of virus in animals is determined by isolation and flourescent and immunoflourescent techniques. Serum levels of hemagglutination inhibition antibodies to both agents are followed. Lymphocyte transformation and cytotoxic activity is studied in spleen cell cultures in the presence of phytohemagglutinin on specific antigens. Any tumors which may develop are studied by light and electron microscopy. Extracts of MVM-containing tumor cells will be injected into newborn mice in addition to being studied in tissue culture.

Major Findings: Attempts to infect embryonic animals by maternal infection in the first week of pregnancy have failed, presumably because infected embryos are reabsorbed. Late gestation maternal infection of 8 animals has resulted in the live birth of 53 animals, 39 of which survived to adult life. All progeny have appeared to be normal, most having been observed for 1 year. Two such animals have died of solid tumors; one of these was lost to study and the other was found to have a tumor in the area of the right uterine horn with hepatic metastases. Extracts of both the tumor and liver yielded a hemagglu-

tinating material presumed to be MVM antigen, suggesting that the animal was either reinfected late in life or had been chronically infected since fetal life. Cell-free materials from these tissues are now being studied both in vivo and in vitro in an attempt to define the possible role of MVM, either directly or indirectly, in the etiology of the tumor.

Significance to Biomedical Research and the Program of the Institute: Infection of the human embryo or fetus with rubella or cytomegalovirus is associated not only with developmental abnormalities and mental retardation, but also with infection persisting for months or years after, despite the presence of neutralizing antibody in the serum. There are several examples of related phenomena in experimental animals infected during prenatal or neonatal life. Previous studies have suggested that an impairment of the immune function of lymphocytes may be associated with such persistent infection. These phenomena provide insight into the question of how viral infection is normally terminated, and represent a challenge to older ideas on the role of specific cellular immunity in the natural history of virus infections. The investigation described represents a direct experimental approach to elucidating the cause and course of chronic infection following initial exposure to the agent during immunologic immaturity. The results of this investigation will be of immediate relevance to our understanding of other types of illness known or suspected to be associated with chronic viral infection. In addition, they may suggest new approaches to research in the role of congenital viral infection as a cause of diseases of unknown etiology such as prematurity, idiopathic growth failure, malignancy, mental retardation, developmental malformation, and autoimmune diseases.

Proposed Course of Project: To be confined at present level of effort.

Honors and Awards: None

Publications: None

Serial No. HD DB - 6
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immune Mechanisms in Experimental Allergic Encephalomyelitis (EAE)

Previous Serial Number: Same

Principal Investigator: Lon R. White, M.D.

Other Investigators: George Nemo, Ph.D.
Marian Kies, Ph.D., Section on Myelin Chemistry, NIMH

Cooperating Unit: Section on Myelin Chemistry, Laboratory of Cerebral Metabolism, DBBR, NIMH
Epidemiology Branch, C & FR, NINDS (corresponding project serial no. NDS(CF)-70 E 1834)

Man Years

Total:	0.20
Professional:	0.15
Other:	0.05

Project Description:

Objectives: To demonstrate in vitro "activation" of lymphocytes from guinea pigs with EAE by a myelin derived basic protein.

Methods Employed: Techniques of lymphocyte cytotoxicity and blastogenic transformation have been employed.

Major Findings: No cytotoxicity has been demonstrated. Transformation has been observed in some, but not all, experiments. These results seem to relate to the following variables: a) the "batch" of basic protein used, b) allotypic identity or dissimilarity between lymphocytes and target cells, c) strain of guinea pig utilized, d)interval between inoculation on animals and testing of lymph node lymphocytes.

Significance to Biomedical Research and the Program of the Institute: EAE is generally held to be pathogenically similar to certain human demyelinating diseases, particularly the post-infections encephalitides and multiple sclerosis, and may involve lymphocyte-mediated autoimmunity. The in vitro demonstration of a basic protein "activatable" lymphocyte would be of great value to a more complete understanding of the experimental disease and would suggest a new approach to the human diseases. The knowledge gained relating to

lymphocyte function would be of immediate relevance to problems of auto-immunity and chronic infection in certain diseases definitely or possibly due to viral infection during prenatal and neonatal life.

Proposed Course of Project: No further experiments planned at this time.

Honors and Awards: None

Publications: None

Serial No. HD DB - 7
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Specific Cellular Immunity in Recovery from Viral Infection

Previous Serial Number: Same

Principle Investigator: Lon R. White, M.D.

Other Investigators: Harrie Anne Sutton, biologist

Cooperating Units: None

Man Years

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To determine the role and mechanism of acquired, specific, cell-mediated immune responses in recovery from viral infection *in vitro* and *in vivo*.

Methods Employed: The initial studies have utilized infection of mice, any guinea pigs, MVM (minute virus of mice) and vaccinia, virus. Spleen cells from control or immunized animals have been put into culture with cells previously infected with the appropriate agent. The following variables are: a) virus replication, b) virus-induced cell injury, c) cell-induced cell injury (lymphocyte cytotoxicity).

Major Findings: None to date

Significance to Biomedical Research and the Program of the Institute: It appears likely that specific cellular immunity may play a major role in termination of viral infections *in vivo* and in the host cell injury seen in certain viral infections. It is further hypothesized that an incompetence or abnormality of this mechanism may occur when the animal is first exposed to the virus during immunologic immaturity. The *in vitro* demonstration of such phenomena would represent a major advancement in our understanding of basic immune phenomena and the pathogenesis of virus-induced diseases.

Proposed Course of Project: To be continued.

Honors and Awards: None

Publications: None

Serial No. HD DB - 15
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Search for a Negative DNA Strand in Cells Infected with the Minute Virus of Mice (MVM).

Previous Serial Number: None

Principle Investigator: Lon R. White, M.D.

Other Investigators: Harrie Anne Sutton, biologist
George Nemo, Ph.D.
Gary Cooper, biologist
Jacob A. Brody, M.D.

Cooperating Unit: Epidemiology Branch, C & FR, NINDS (corresponding project serial no. NDS(CF)-71 E 1922)

Man Years

Total:	0.4
Professional:	0.4
Other:	0.0

Project Description:

Objectives: a). To demonstrate a "template" DNA (negative strand) complementary to the single stranded DNA (positive strand) of the MVM virion. b). To determine if the negative strand is associated with the cell's chromosomal DNA. c). To investigate the kinetics of negative strand synthesis relative to cellular DNA replication, cellular division, virus infection, and virus production.

Methods Employed: These studies will utilize H^3 or C^{14} "tagged" viral DNA with annealing demonstrated by: a) liquid scintillation detection of retained isotope on a filter, b) autoradiography with virus infected cells in metaphase. Some experiments will use purified chromosomes and chromosomal DNA from mitotically phased cells in culture.

Major Findings: None to date

Significance to Biomedical Research and the Program of the Institute: Characterization of virus-cell interactions using a single-stranded DNA virus (parvovirus group, of which MVM is representative) is expected to advance our understanding of the mechanisms by which a virus infection might alter the genetic potential of the host cell (either a somatic or germ cell) as well

as of mechanisms involved with the establishment of latent and chronic viral infections. These may be directly relevant to the etiologies of certain reproductive, developmental, and chronic diseases whose causes are now unknown.

Proposed Course of Project: To be continued at an increased level of effort.

Honors and Awards: None

Publications: None

Serial No. HD DB - 16
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development and Application of Methods for the Study of the Minute Virus of Mice (MVM).

Previous Serial Number: None

Principal Investigator: Lon R. White, M.D.

Other Investigators: Harrie Anne Sutton, biologist

Cooperating Unit: None

Man Years

Total:	0.00
Professional:	0.80
Other:	0.00

Project Description

Objectives: To develop better methods for the propagation, purification, detection and radioisotope "tagging" of MVM. To apply these to an investigation of the basic properties of this agent, including studies on its "growth" *in vitro*, its stability (heat, pH, freezing, VIV irradiation) and its composition.

Methods Employed: The agent is grown in tissue culture and detected by CPE production, hemagglutination, and immunofluorescence. A variety of primary and continuous tissue culture cell lines are used, as well as two strains of the virus. The virus and its DNA is purified by ultracentrifugation. Radioisotope "labeling" is carried out both *in vitro* and *in vivo*.

Major Findings: Previous reports have described the growth of MVM in only two cell lines, both primary, with CPE in only one of these. We have extended this to 4 continuous cell lines, 2 of which show CPE. We have shown that whereas virus production demands that the host cell divide, infection can occur in non-dividing cells and the virus can remain latent without apparent injury to the cell until it divides. In addition, we have developed a cell line which has been producing virus in high concentration continuously for 4 months without apparent adverse effects on the host cell. We have accomplished a 100-1000 x improvement in yield of virus and hemagglutinating antigen and have shown that purification by isopycnic ultracentrifugation is associated with moderate virus disruption and loss of infectivity. Additional studies have revealed no significant loss of hemagglutination or infectivity titers with

3 cycles of freeze-thawing or with heating at 56°C for up to 90 minutes. UV inactivation progresses in approximately one $\log_{10}$ decrements. The in vitro infectivity titration method has been improved from a rather unreliable one month procedure (which works poorly with "wild" strains of MVM) to a sharply reproducible, reliable one week procedure usable with all strains. A method is being developed to label viral DNA in vitro (with H^3 or C^{14} dimethl sulfate) which yields a high specific activity. In vivo labeling with H^3 thymidine has also yielded DNA of moderate specific activity. Preliminary results indicate that there is no DNA polymerase (DNA dependent) associated with purified MVM and that the virus possesses only a single protein.

Significance to Biomedical Research and the Program of the Institute: MVM is representative of a group of small, single-stranded DNA viruses (the parvoviruses) which are characterized by ubiquity, generally low virulence and cytopathogenicity, an apparent preference for dividing cells, and a high frequency of transplacental infections. The study of this agent is expected to lead to a better understanding of mechanisms and the possible role of viruses in the causation of certain genetic, reproductive, and developmental abnormalities. MVM has been relatively unstudied, presumable because of technical problems. The improved methods which we have developed, and the basic information obtained by the application of these methods now make such studies feasible.

Proposed Course of Project: To be continued at present level of effort.

Honors and Awards: None

Publications: None

Serial No. LEP/NIAMD - 17
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the Relationship of Chromosomal Abnormalities and Certain Viral Infections in Mice. (Alternate title: Effects of Viruses on Mitosis and Meiosis in the Mouse.)

Previous Serial Number: Same

Principal Investigators: Beverly J. White, M.D., LEP, NIAMD
Lon R. White, M.D.

Other Investigators: George Nemo, Ph.D.
Jacob A. Brody, M.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMD
Developmental Biology Branch (Unit on Chronic and Congenital Viral Infections), NICHD

Man Years

Total:	.50
Professional:	.50
Other:	.00

Project Description

Objectives: a). To localize within dividing mouse spleen cells in vitro, particularly in relation to chromosomes, the site of residence of the infecting viral genome (of the minute virus of mice - MVM) and the MVM template DNA. b). To search for evidence of injury by virus to chromosomes of spermatogonia and somatic cells. c). To determine whether perinatal infection results in infection of the germ cells and, if so, to determine the duration of infection as well as the chromosomal and reproductive consequences of such infection.

Methods Employed: The viruses under study are two strains of MVM and two strains of reovirus type 1. The animal used is the Cumberland View Farms C57BL/6 mouse. The viruses are "grown" and quantitated in tissue culture. Intracellular MVM genome (infectious or template DNA) is localized by autoradiography using tritium "tagged" MVM DNA. Chromosome studies are carried out on short term spleen cell cultures as well as on cytologic preparations made directly from the spleen, testes, and bone marrow of infected and control mice. Viral antigen is localized using immunofluorescent and immunohistochemical methods.

Major Findings: a). A "fate" study in mouse spleen cells using tritium-labeled virus preparations showed no difference in autoradiographic grain localization between control and virus infected cultures. These were carried out with "tagged" viruses of relatively low infectivity and specific activity (radioactivity). b). Meiotic preparations from the testes of adult mice were studied 4 days, 1 week, 3, 6, and 12 weeks following MVM infection. The only notable observation was of a relative decrease in number of certain cell types, suggesting an effect on the course of spermiogenesis.

Major improvements in methods involved with the propagation and quantitation of MVM have been accomplished. These have been used to produce viral "reagents" to be used in additional experiments.

Significance to Biomedical Research and the Program of the Institute: The role of viruses in the etiologies of chromosomal and developmental diseases is widely discussed, but negligibly investigated. The interaction of non-cytolytic, non-oncogenic viruses with chromosomes and the spindle is also essentially undefined. The potential of these investigations is vast; basic information may be gained important to an understanding of human disease pathogenesis as well as to current concepts on the evolution of viruses and the basic nature of their interactions with dividing, differentiating cells.

Proposed Course of Project: To be continued at same level of effort.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 71 E 1921
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Measles infection of mice: Effect of maternal immunity.

Previous Serial Number: None

Principal Investigators: Jacob A. Brody, M.D.
George Nemo, Ph.D.

Other Investigators: Lon R. White, M.D., DB, NICHD
Gary Cooper, Biologist

Cooperating Unit: None

Man Years:

Total:	.20
Professional:	.20
Other:	.00

Project Description

Objectives: To develop an experimental animal model to study the pathogenesis of subacute sclerosing panencephalitis (SSPE).

Methods Employed: Adult female mice previously immunized with measles virus will be bred and their offspring challenged with measles at various times after birth. Measles antibody levels will be determined periodically and a thorough analysis of mouse tissues for measles virus and measles-related antigens will be performed.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: An agent which by most virological criteria resembles measles virus has been isolated from brains of SSPE patients. A papova-like virus has been visualized in electron micrographs of specially treated cell lines innoculated with SSPE brain material suggesting the possibility of a dual infection. The exact role these agents play in the pathogenicity of the disease is unclear. An experimental animal model may aid considerably in elucidating the mechanism.

Proposed Course of Project: See "Methods Employed"

Honors and Awards: None

Publications: None

Serial No. HD DB 12(c)
1. Developmental Biology Branch
2.
3. Stanford, California

NIH-PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Preparation of a Radiographic Standard of Reference of the Growing Hand and Wrist for the United States National Health Examination Survey.

Previous Serial Number: Extension of HD DB 12(c)

Principal Investigator: William W. Greulich, Ph.D.

Other Investigators: None

Cooperating Units: Arthur J. McDowell, Director
Division of Health Examination Statistics
National Center for Health Statistics

Dr. S. Idell Pyle
Case-Western Reserve U.
Cleveland, Ohio

Depts. of Anatomy and Pediatrics
Stanford University School of Medicine
Stanford, California

Project Description:

Methods Employed: A systematic comparison of the skeletal status of the right and left hands of the same children, using for the purpose hand-radiographs collected earlier.

Major Findings: Occasional consultant and collaborator with the National Center for Health Statistics in their very extensive survey of the physical growth and development of a representative sample of children from all parts of the United States. When completed, the survey will include the largest number of children so extensively studied in this country.

Since a hand-radiograph is made of every child examined, the data from this survey will enable us to determine the extent to which the growth and developmental status of the children of this country have changed during the thirty-odd years that have passed since we studied the children on whom the Greulich-Pyle standards of skeletal development are based. This information will indicate the extent, if any, to which the chronological age equivalents of the standards in our Atlas need to be modified in order to better fit the present-day children of this country.

During the current year, collaborated with Dr. S. Idell Pyle in preparing the book listed under publications.

Proposed Course of Project: None. This researcher is anticipating retirement and may continue this project on his own resources.

Honors and Awards: None

Publications: Pyle, S. I., Waterhouse, A. M. and Greulich, W. W. A Radiographic Standard of Reference of the Growing Hand and Wrist for the United States National Health Examination Survey. Cleveland, Case-Western Reserve Univ. Press, (in press).

Serial No. HD DB 4(9)(c)
1. Developmental Biology Branch
2.
3. Stanford, California

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: An Anthropometric Study of American-born Japanese Adults

Previous Serial Number: Same

Principal Investigator: William W. Greulich, Ph.D.

Other Investigators: None

Cooperating Units: Departments of Anatomy and Pediatrics
Stanford University School of Medicine
Stanford, California

School of Public Health
University of California
Berkeley, California

Atomic Bomb Casualty Commission, Hiroshima

Project Description:

Objectives: To determine the terminal adult stature and some other bodily dimensions and proportions of young American-born Japanese men and women on whom similar measurements and hand-radiographs were made twelve years ago. A comparison is being made of the adult size and bodily proportions of these American-born Japanese with those of Japanese of the same age and sex in Japan. This follow-up study was begun in California in the summer of 1968.

Methods Employed: Locating and re-examination of these subjects has continued to be difficult and time-consuming. The increasing race-consciousness of many of the local Japanese, especially of the men, has not made the task any easier.

The Atomic Bomb Casualty Commission in Hiroshima has provided useful supplementary data on the stature of native Japanese of the same age-range as our subjects.

The School of Public Health of the University of California at Berkeley has provided access to records of a recent nutritional study. Stature and weight data on a large number of California Japanese adults will serve to augment present data and aid in their evaluation.

Proposed Course of Project: Completion this year.

Honors and Awards: None

Publications: None

Serial No. HD DB 3(c)
1. Developmental Biology Branch
2.
3. Stanford, California

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Form and Incidence of Dysplastic Middle Phalanges of the Fifth Fingers among Caucasians with Down's Syndrome and Mentally Normal Japanese.

Previous Serial Number: Same

Principal Investigator: William W. Greulich, Ph.D.

Other Investigators: None

Cooperating Units: Departments of Anatomy and Pediatrics
Stanford University School of Medicine
Stanford, California

Project Description:

Methods Employed: To determine the type and incidence of dysplastic middle phalanges of the fifth fingers among Caucasians with Down's syndrome and mentally normal Japanese. During the current year this investigation has been extended to include mentally normal Negroes and both mongoloid and Negro subjects with Down's syndrome.

Major Findings; In the earlier study it was found that both the mentally normal Japanese and Caucasians with Down's syndrome had a very high incidence of dysplastic middle phalanges of the fifth fingers, but that the type of dysplasia was quite different in the two groups. In an effort to determine what type of dysplastic phalanges, if any, occurred in Japanese, Chinese, and American Indian Down's syndrome subjects, it was found that, without exception, those who had dysplastic phalanges had the same type that occurs so frequently among Caucasian mongoloids. None of them had the type that is found so commonly among mentally normal individuals of their race.

The current study included, too, an examination of hand-radiographs of several hundred mentally normal Negroes and of a few Negro mongoloids. This defect was found to be very rare among normal Negroes; indeed, no clear example was found in the films studied. In one Negro mongoloid, however, the same type of phalangeal dysplasia that is seen among Caucasians and Orientals with Down's syndrome was found.

Proposed Course of Project: Completed. One further publication is in preparation.

Honors and Awards: None

Publications: Greulich, W. W.: The incidence of dysplasia of the middle phalanx of the fifth finger in normal Japanese, in some American Indian groups, and in Caucasians with Down's syndrome. In Kretchmer, N. and Walcher, D. N. (Eds.) Environmental Influences on Genetic Expression, Washington, D.C., U.S. Government Printing Office, 1970, pp 91-103.

Serial No. HD DB1(c)
1. Developmental Biology Branch
2.
3. Stanford, California

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A Case of Like-sexed Dizygotic Twins, Both of Whom are Mongoloid.

Previous Serial Number: Same

Principal Investigator: William W. Greulich, Ph.D.

Other Investigators: Herman Yannet, M.D., Medical Director
Southbury Training School, Southbury, Connecticut
Chris Plato, Ph.D., CDSB, NICHD, Bethesda, Maryland

Project Description:

Objectives: To illustrate that the presence or absence of dysplastic middle phalanges of the fifth fingers in individuals with Down's syndrome is not necessarily related to the severity of the other stigmata of that syndrome.

Methods Employed: Review of case history, photographs, palm-and finger-print, assays of their haptoglobins.

Major Findings: Have succeeded in obtaining additional, pertinent information on these twins from the Southbury Training School. The completed report is in process of publication.

Proposed Course of Project: None

Honors and Awards: None

Publications: A case of like-sexed dizygotic twins, both of whom are mongoloid, has been submitted to Journal of Mental Deficiency Research.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Reproduction Research Branch

SUMMARY

In July, 1970, the Endocrinology Branch of the National Cancer Institute became the Reproduction Research Branch of the National Institute of Child Health and Human Development. The Branch was strengthened by the addition of the Sections of Endocrinology and Reproductive Anthropometry. The strengths and skills of the scientists in all the groups are complementary; thus there is extensive collaboration among the senior investigators.

The mission of the Reproduction Research Branch is the development of scientific data and theory basic to reproductive biology. Research efforts therefore extend from the chemistry of the protein and steroid hormones to physiologic and clinical studies in man. To prosecute these investigations successfully a variety of tools and skills are necessary: physical chemistry of macromolecules, protein isolation and analysis, tissue and organ culture, radioligand assays of protein and steroid hormones, mathematical analyses of immunoassays and the kinetics of protein hormone binding, characterization of in vitro steroid biosynthetic pathways, dynamic analysis of steroid hormone metabolism in man. A continuing interest and involvement in clinical problems is not only desirable, but necessary since the results of study in man are the final arbiter of the relevance of results in the laboratory.

The group in protein chemistry has continued its development of better techniques for the isolation of proteins and polypeptides. They have described macromolecular mapping using preparative polyacrylamide gel electrophoresis to facilitate isolation of polypeptides from mixtures. Their collaboration with investigators in the Branch, and within and outside the National Institutes of Health has resulted in significant advances in several areas. The expertise developed here has been recognized by major reviews of these techniques in "Science" and in "Analytic Biochemistry".

The tissue culture laboratory has been able to resolve a fundamental question about gonadotropic hormones. They have shown that gonadotropin heterogeneity is due, in large part, to the synthesis of gonadotropins with different sialic acid content. In other studies, they have formed viable hybrids of human trophoblasts with specific lines of mouse and human cells. Since the trophoblasts have four metabolic markers and several distinctive marker chromosomes, the system will be useful for studying gene function in mammalian cells.

Continuing work initiated last year, it has been shown that the sialic acid moiety of the glycoprotein hormones, the gonadotropins, is the major determinant of their clearance rate. These studies also resulted in a new technique for labeling gonadotropins without loss of biologic activity. The roles of the polypeptide sub-units of HCG in its biologic activity and immunogenicity have been clarified.

The binding of gonadotropins to receptors in the ovary and testis has been a focus of interest. The binding of FSH to the estrogen-stimulated ovary of the rat was described. Luteinizing hormone and HCG were shown to be bound with high affinity using intact and broken cell preparations from rat testis. In this system, binding depended on the specific polypeptide chain with no contribution from the carbohydrate fraction. It has also been possible to follow testosterone production by the Leydig cells *in vitro* in the same system.

The pace of clinical investigation has continued and this year we established male and female infertility clinics in addition to our Endocrinology Clinic. The first evidence has been obtained that, in man, germinal epithelium activity modulates the release of FSH, a most important finding for studies of control of spermatogenesis.

Our earlier findings regarding the genesis of the short luteal phase have been confirmed by experimental production of this phenomenon in women. These data have suggested methods to treat this type of infertility and appropriate clinical trials are under way. In continuation of earlier studies of the dynamics of estrogen metabolism, we have found that the estrogen in highest concentration in blood is estrone sulfate. The biologic activities of this estrogen are of potential importance in estrogen effects.

This year as in previous years we have been aided greatly by several guest workers and visiting scientists. Their skills and fresh outlooks have stimulated us and, in turn, they have benefitted from the several ongoing studies. Their names are appended. In addition to these workers, we have continued to host many technicians and professionals who wish to learn one or more techniques used in our laboratories.

Guest Workers FY 1971
Length of Stay and Place of Origin

J. A. Bermudez, M.D.	Institute of Nutrition Mexico City, D. F.	3 years
G. G. Kapadia, Ph.D.	Bombay University Bombay, India	3 years
Howard Brinkley, Ph.D.	University of Maryland College Park, Maryland	6 months
Joel Menard, M.D.	Hopital Broussais Paris, France	4 months
Tsuneo Tsuruhara, M.D.	Yodogawa Christian Hospital Osaka, Japan	2 years
Yukitaka Miyachi, M.D.	University of Tokyo Tokyo, Japan	1 year
Eberhard Nieschlag, M.D.	Med. Univ.-Klinik u Poliklinik Mainz, Germany	1 year
J. L. Vaitukaitis, M.D.	Boston University School of Medicine Boston, Massachusetts	1 year

Serial No. RPL-1
1. Reproduction Research Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biology of Reproduction: Clinical and Experimental

Principal Investigator: Dr. Mortimer B. Lipsett

Other Investigators: Dr. G. T. Ross, Dr. P. O. Kohler, Dr. C. E. Cargille, Dr. William Bridson, Dr. R. J. Sherins, Dr. David Rodbard, Dr. Andreas Chrambach, Dr. Kevin Catt, Dr. Maria Dufau Catt, Dr. J. A. Bermudez, Dr. Howard Kulin, Dr. Edward Reiter, Dr. Robert Goldenberg, Dr. Jay Grodin, Dr. David Van Thiel, Dr. James Hammond, Dr. Henry Ruder, Dr. D. Lynn Loriaux, Dr. Elseworth Philip, Mr. P. E. Rayford, Mrs. Joyce Kingfield, Mr. James Brice, Mr. David Ryan, Mr. Grant Coffman, Mrs. Mary Wyckoff and Mr. Rudolph Reid.

Cooperating Units: Solid Tumor Service, National Cancer Institute; Mathematical Research Branch, National Institute of Arthritis and Metabolic Diseases; Laboratory of Biochemistry and Metabolism, National Institute of Arthritis and Metabolic Diseases; Laboratory of Cell Biology, National Cancer Institute; Office of the Clinical Director, National Institute of Child Health and Human Development.

Man Years:

Total: 43.5
Professional: 27.5
Other: 16

Project Description:

Objectives:

1. To understand the mechanisms of regulation of gonadal secretion.
2. To define the regulatory processes involved in maintenance of blood and tissue hormone levels.
3. To analyze the events of the normal and abnormal menstrual cycle.
4. To explore the control of spermatogenesis.
5. To study and attempt to modify the side-effects of estrogens.

Methods Employed:

These vary widely since the research encompasses such methodologic groups as protein chemistry, mathematical biology, tissue culture, steroid and protein hormone analysis, and clinical investigation. The investigators have been eclectic in their choice of methods and have adapted old methods or developed new ones for special needs. Some of the important new techniques are presented below:

Protein chemistry: The group has developed an apparatus for preparative polyacrylamide gel electrophoresis of proteins using two-phase fractionation. They have prepared highly crosslinked polyacrylamide gels suitable for fractionation of macromolecules in the multimillion molecular weight range.

Mechanism of Action: In collaboration with the Laboratory of Biochemistry and Metabolism, a method for labeling the gonadotropins with tritium was devised. Antisera have been prepared against gonadotropin subunits to enable study of the components of the gonadotropins. A sensitive system has been used to measure binding of gonadotropins to the testis and the effect of LH on testicular hormone secretion in vivo.

Analytic techniques: Radioligand assays have been adapted for the measurement of a new estrogen in blood. Specific antisera that distinguish between LH and HCG have been obtained by immunization with specific subunits.

Major Findings:

Protein chemistry: There have been important new techniques developed during this year. Macromolecular mapping of multicomponent systems has been achieved with modified preparative gel electrophoresis. A theory of resolution of multicomponent systems has been proposed.

Conditions for achieving polymerization efficiency and optimal initiator concentrations for the polymerization of acrylamide have been determined. The redox potential within polyacrylamide gels has been quantified to arrive at a method to generate reducing conditions in the gel. An electrophoretic concentration device to achieve near-quantitative recovery from gels has been developed.

Highly crosslinked polyacrylamide gels have been prepared that are suitable for fractionation of molecules in the multimillion MW range. Retardation coefficients in these gels have been measured for a series of globular and of linear molecules in order to prove the theoretically predicted possibility that these gels can be used for the determination of molecular volume.

The use of the techniques developed in this laboratory has been essential for the resolution of several important biologic problems. The

molecular weight and charge of human prolactin have been measured for the first time, and its separation from the most abundant species of growth hormone has been achieved. Rat serum prolactin has been characterized similarly.

Human chorionic gonadotropin from tissue culture of human trophoblast was isolated. Other protein hormones characterized and separated by gel electrophoresis and isoelectric focussing are: human FSH, human urinary LH in the presence of HCG, and human immunoreactive insulins.

Embryonic rabbit hemoglobin was shown to differ from fetal hemoglobin, and its time course of development has been traced. Mouse antitrypsin has been isolated.

Mathematical Biology: The theory of radioligand assays has been examined intensively. In this field we have developed the following:

1. A theory of kinetics (transient state) of radioimmunoassays. This has lead to development of methods for improvement of assay sensitivity.

2. The solution of a comprehensive theory of the problem of radioimmunoassays involving any number of ligands, and any number of binding proteins.

3. An exact theory for prediction of the error around a radioimmunoassay dose response curve. This theory has also been tested by Monte Carlo methods.

4. Computer programs and mathematical methods for the estimation for the parameters of real radioimmunoassay systems.

5. Computerization of a statistical quality control procedure, developed in this laboratory several years ago.

6. A statistical method and computer program for obtaining relative potency estimates when two preparations are studied at multiple dose levels. This program also includes a test for parallelism.

7. A computer program to optimize radioligand assay conditions to obtain the best precision at any desired point on the dose response curve.

8. A computer program to optimize assay conditions in terms of the molar concentration of labeled ligand and molar concentration of binding sites, in order to minimize the least detectable dose.

9. A theory of the kinetics of the separation of bound and free forms of radioimmunoassays.

10. A mathematical theory of immunoradiometric assays (involving the use of a labeled antibody rather than the use of a labeled antigen).

To improve the practice of gel electrophoresis and protein fractionation, the following mathematical techniques have been developed:

1. An exact theory of pore gradient electrophoresis (confirming and extending the previous theory which did not include the effect of diffusion).

2. A computer program for optimization of conditions to optimize resolution between any two species.

3. Statistical techniques and computer programs for testing identity of proteins studied by polyacrylamide gel electrophoresis (involving joint 95% confidence limits for the retardation coefficient and free mobility.

Mechanism of Action: Desialylation of HCG increased the rate of metabolism thereby explaining differences in biologic activity of several species of HCG. Methods developed in these studies were used to introduce tritium into HCG and FSH without altering biologic or immunologic activity and thus providing an important research tool.

Desialylation of FSH and LH has been accomplished similarly. There was marked loss of biologic activity in standard bioassays, but an enhancement of immunogenicity.

Reversible and specific binding of ^{125}I-labeled gonadotropins has been demonstrated to intact cells and sub-cellular particles of testis. Particulate fractions from broken cell preparations of the rat testis and the pseudopregnant rat ovary have been employed as the basis of radioligand-receptor assays for LH and HCG. Such assays have a working range of 5-500 MU of LH and HCG, providing a sensitive system for measurement based on a 'biological' property of the tropic hormones and have cross-species application to assay of gonadotropins.

The binding of ^{125}I tracer HCG by testis and ovarian receptor fractions is inhibited as effectively by desialylated forms of LH and HCG as by the intact molecules, indication that the sialic acid residues are not essential for receptor-hormone combination. Studies in activation of adenyl cyclase in the testis and ovary by gonadotropins are being performed to determine whether a relationship between hormone binding, cyclase activation and steroidogenesis can be established.

The chorionic gonadotropin synthesized by clonal lines of human trophoblast has been examined by polyacrylamide gel electrophoresis and isoelectric focussing. The hormone synthesized by the cell lines has a molecular weight similar to that of highly purified HCG extracted from human pregnancy urine. However, the net charge and isoelectric points differ.

Furthermore, the hormone produced in culture is heterogeneous with regard to isoelectric point and differences exist among the various clones. These changes suggest that the cells synthesize gonadotropin molecules with differing amounts of sialic acid.

The chorionic gonadotropin synthesized in culture has been shown to be biologically active in two bioassay systems. Biological activity was compared with potency estimated by radioimmunoassay and significant differences in bio-immuno potency ratios were found among chorionic gonadotropins produced by different cell lines. Additionally, bio-immuno potency ratios of chorionic gonadotropin produced in culture differed from those of chorionic gonadotropins extracted from the urine of pregnant women. It is suggested that previously observed differences in bio-immuno potency ratios for urinary extracts of human chorionic gonadotropins arise at the cell of origin.

The interstitial cell fraction of the testis was unresponsive to gonadotropins in vitro, and the response was not restored by recombination with the tubule fraction, demonstrating that normal structural relationships of the testis are essential for optimum testosterone synthesis and stimulation by gonadotropin. The intact decapsulated testis responded to as little as 1 mU of HCG and 0.3 mU of ovine LH with 100% increases in testosterone synthesis, and gave a dose-response curve with added HCG over the range 1-10 mIU. The sensitivity of the in vitro rat testis preparation to trophic hormones provides a valuable system for studying the effects of structural modifications on hormone action and the relative biological activities of gonadotropins at the target cells.

Methods for measurement of renin substrate, activity and concentration in rat plasma have been developed and used in studies on the effects of estrogen upon the renin-angiotensin system. Evidence of rapid and early stimulation of renin substrate formation has been obtained with increased plasma levels of the protein as soon as five hours after estrogen administration.

Gametogenesis: Studies of men who have been treated for Hodgkin's disease have shown conclusively that those men with azoospermia have complete absence of germinal elements on testicular biopsy and elevation of FSH secretion; in contrast, those patients with sperm in the ejaculate have normal testicular biopsies and FSH secretion is normal. Since in both groups the pituitary-Leydig cell axis is normal, this is firm evidence for the presence of specific germinal epithelial factors that regulate FSH release. This is the first conclusive evidence in man relating the control of FSH secretion to germinal epithelium function.

The effect of estrogen on follicular development was initiated by extending older observations that estrogen stimulates follicular maturation in hypophysectomized immature rats. This was found to be due to granulosa cell hyperplasia and that estrogen sensitized the follicle to FSH stimulation. Tritium-labeled FSH could then be shown to localize in the follicle.

Clinical Investigation:

When the early FSH peak was abolished by administration of estrogen to normal women, the LH peak did not occur and the menstrual cycle was shifted. This is further evidence that the early secretion of FSH is related to follicular development. This effect of estrogen could be overcome by simultaneous administration of clomiphene citrate demonstrating further that this agent acts as an anti-estrogen. Many of these women had short luteal phases accompanied by lower than normal FSH levels, thus supporting our concept that the inadequate luteal phase results from an inadequate secretion of FSH.

On the basis of previous work, it was postulated that there was an estrogen compartment that slowly exchanged with plasma estrone and estradiol. This compartment has been identified as estrone sulfate. Methods were developed to quantify this steroid, and it was shown that estrone sulfate is the most abundant estrogen in blood and that it originates from plasma estrone and estradiol. Based on studies of the conversion of estrone sulfate to estrone and estradiol, it seems likely that estrone sulfate exerts some estrogenic effect *per se*.

We have attempted to examine pathways of steroid biosynthesis in human adrenal cortex *in vivo*. When ACTH was infused, there was stimulation of the Δ^5-pathway during the first twenty minutes as shown by marked increases in plasma concentrations of pregnenolone and 17-hydroxypregnenolone when compared with the increments in plasma progesterone, 17-hydroxyprogesterone, and cortisol. These are the first *in vivo* data in man relevant to early steroid biosynthetic pathways.

Significance to Bio-Medical Research and to the Institute:

The data recorded here are basic to an understanding of many phenomena in reproductive biology. The emphasis on structure and mode of action of the gonadotropins is justified in view of their roles as regulators of gonadal function.

The quantitative analysis of the hormonal events of the menstrual cycle enables the clinical investigator to detect perturbations of the cycle that are either pathologic or are imposed by use of specific agents. Continuing work in this area has led to identification of estrogens present at high concentration in blood with potent biologic activity. In order to test or devise contraceptive regimens in women, the precise measurement of the hormonal milieu will continue to be necessary. The work already has practical consequences; the cause of a type of sterility has been identified and treatment suggested, and a means of examining corpus luteum function during early pregnancy was found.

Proposed Course:

Studies of binding of polypeptide hormones to membrane receptors in ovary and testis will be important in the coming year. These studies are of

fundamental importance in biology and also have the potential of revealing methods by which gonadal function can be altered.

A larger effort will be made in the field of gametogenesis. Both clinical and experimental studies will be carried out to assess the role of FSH in gametogenesis and to describe the signals for the FSH response. The growing male and female infertility clinics will contribute much of the human study subjects.

Particular side effects of the estrogenic component of the "pill" will be studied with emphasis on hypertension. With some expansion of our laboratory capacity for routine measurements, it is intended to engage in wider screening efforts with appropriate long-term surveillance of the population.

Honors and Awards: Mortimer B. Lipsett

Elected to Association of American Physicians, May, 1970.

Appointed member of American Cancer Society, Study Section on Biochemistry and Chemical Carcinogenesis, June, 1970.

Member, American Board of Internal Medicine, Endocrine Test Committee, 1970-1973.

Member, Program Organizing Committee, IV International Endocrinology Congress, 1971.

Invited Plenary Lecture, 3rd International Congress on Hormonal Steroids, Hamburg, Germany, September, 1970.

Invited Lecturer, 22nd Annual Postgraduate Assembly, Endocrine Society, St. Louis, October, 1970.

Invited Lecturer, Seventh Annual Clinical Day, Jewish General Hospital, Montreal, Canada, October, 1970.

Participant, President's Science Advisory Committee, December, 1970.

Visiting Professor, Southwestern Medical School, Dallas, Texas, March, 1971.

Faculty Member, Postgraduate Seminar in Cancer, University of Texas Medical School, San Antonio, Texas, March, 1971.

Invited Lecturer, 2nd National Conference on Breast Cancer, Los Angeles, May, 1971.

Invited Faculty Member and Lecturer, 6th Mexican Congress of Gynecology and Obstetrics, Mexico City, June, 1971.

Honors and Awards: G. T. Ross

Invited Lectures:

Conference on The Regulation of Mammalian Reproduction, NIH, Bethesda, Maryland, September, 1970.

Visiting Professor, Peter Bent Brigham Hospital, Boston, Mass., October, 1970.

The University of Michigan, Ann Arbor, Michigan, November, 1970.

College of Physicians and Surgeons, New York City, December, 1970.

George Washington Hospital, Washington, D.C., January, 1971.

Walter Reed Medical Center, Washington, D.C., February, 1971.

Washington Hospital Center, Washington, D.C., February, 1971.

Arlington Hospital, Arlington, Virginia, February, 1971.

Sixth Annual Cancer Symposium, Akron City Hospital, Akron, Ohio, March, 1971.

Plenary Lecture, American Fertility Society, New Orleans, Louisiana, March, 1971.

Endocrine Society Workshop on Competitive Binding Assays, Los Angeles, California, March, 1971.

Northwestern University, Chicago, Illinois, April, 1971.

University of Wisconsin, Madison, Wisconsin, May, 1971.

International Symposium on Gonadotropins, New York Hospital, New York City, June, 1971.

Honors and Awards: Andreas Chrambach

Editorial Board of Analytical Biochemistry, 1970-

Invited Lecturer, Conference on Soluble Antigens, National Institutes of Health, October, 1970.

Invited Lecturer, University of Virginia, Biochemistry Department, March, 1971.

Honors and Awards: Peter O. Kohler

Invited Lectures:

Georgetown University School of Medicine, Washington, D. C., November, 1970.

Vanderbilt School of Medicine, Nashville, Tenn., November, 1970.

Third Karolinska Symposium on Research Methods in Reproductive Endocrinology. Geneva, Switzerland, January, 1971.

Conference on Glycoproteins with Hormonal Activity, Fogarty International Center, National Institutes of Health, April, 1971.

Session in Depth on Hormones at Tissue Culture Association, Lake Placid, New York, June, 1971.

Honors and Awards: David Rodbard

Invited Lectures:

Seventh International Biometrics Conference, Hannover, Germany, August, 1970.

University of Pittsburgh Medical School, Pittsburgh, Pa., Sept., 1970.

Gas Chromatography Club, Greater Washington Area, October, 1970.

Harbor General Hospital, Torrance, Calif., April, 1971.

International Atomic Energy Agency, Training Course on Radioimmunoassay Techniques, Pisa, Italy, May, 1971.

Honors and Awards: Richard J. Sherins

Invited Lectures:

Veterans Administration Hospital, Los Angeles, Calif., August, 1970.

University of Southern California, Berkley, California, August, 1970.

Urologic Society, Washington, D.C., January, 1971.

Honors and Awards: Jose A. Bermudez

Invited Lectures:

Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania, May, 1971.

Honors and Awards: Kevin J. Catt

Invited Lectures:

Department of Medicine, McMaster University, Ontario, Canada, February, 1971.

Veterinary Research Group, Beltsville, Maryland, March, 1971.

International Atomic Energy Agency Course in Radioimmunoassay Techniques, Pisa, Italy, May, 1971.

College de France, Paris, France, May, 1971.

Publications:

Protein Chemistry

Cantz, M., Chrambach, A., and Neufeld, E. F.: Characterization of the factor deficient in the Hunter syndrome by polyacrylamide gel electrophoresis. Biochem. Biophys. Res. Com. 39: 936-942, 1970

Bridson, W. E., Ross, G. T., and Kohler, P. O.: Immunological and biological activity of chorionic gonadotropin synthesized by cloned choriocarcinoma cells in culture. J. Clin. Endocr., in press

Bermudez, J., and Corvol, P.: Estudio de las caracteristicas fisicas del transcortin. Proceedings of Soc. Mex. Nutr. y Endocrinologia, Acupulco, Mexico, December 1970, pp. 599-614

Corvol, P., Rodbard, D., Bardin, C. W., and Chrambach, A.: Physical properties determined by polyacrylamide gel electrophoresis and binding capacity of testosterone-estradiol binding globulin in human plasma. J. Biol. Chem., in press

Chrambach, A., and Rodbard, D.: Polyacrylamide gel electrophoresis. Science, in press

Doerr, P., and Chrambach, A.: Anti-estradiol antibodies: isoelectric focusing in polyacrylamide gel. Anal. Biochem., in press

Finlayson, G. R., and Chrambach, A.: Isoelectric focusing in polyacrylamide gel and its preparative application. Anal. Biochem., in press

Jovin, T. M., Dante, M. L., and Chrambach, A.: Multiphasic Buffer Systems. Systems Output, Tape 1 through 4, PB196085 to 196088. U. S. Department of Commerce, National Technical Information Service, Springfield, Va.

Jovin, T. M., Dante, M. L., and Chrambach, A.: Multiphasic Buffer Systems. Instructions for Use of the Systems Output, Tape 1 through 4, PB196089. U. S. Department of Commerce, National Technical Information Service, Springfield, Va.

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Jovin, T. M., Dante, M. L., and Chrambach, A.: Multiphasic Buffer Sytems. Instructions for Use of the Systems Catalogue, PB196091. U. S. Department of Commerce, National Technical Information Service, Springfield, Va.

Kohler, P. O., Bridson, W. E., and Chrambach, A.: Human growth hormone produced in tissue culture: characterization by polyacrylamide gel electrophoresis. J. Clin. Endocr. 32: 70-76, 1971

Lunney, J., Chrambach, A., and Rodbard, D.: The factors affecting resolution, band width, number of theoretical plates, and apparent diffusion coefficients in polyacrylamide gel electrophoresis. Anal. Biochem. 40: 158-173, 1971

Maffezzoli, R., Kaplan, G. N., and Chrambach, A.: Physical characterization by polyacrylamide gel electrophoresis of human follicle stimulating hormone from urinary extracts of children, men, pre- and postmenopausal women. J. Clin. Endocr., in press

Maffezzoli, R., Kaplan, G. N., and Chrambach, A.: Fractionation of immunoreactive human chorionic gonadotrophin and luteinizing hormone by isoelectric focusing in polyacrylamide gel. J. Clin. Endocr., in press

Rodbard, D., and Chrambach, A.: Estimation of molecular radius, free mobility, and valence using polyacrylamide gel electrophoresis. Anal. Biochem. 40: 95-134, 1971

Rodbard, D., Kapadia, G., and Chrambach, A.: Pore gradient electrophoresis. Anal. Biochem. 40: 135-151, 1971

Ugel, A. R., Chrambach, A., and Rodbard, D.: Fractionation and characterization of an oligomeric series of bovine keratohyalin by polyacrylamide gel electrophoresis. Anal. Biochem., in press

Mathematical Biology

Feldman, H., Levine, D., and Rodbard, D.: Theory of complex ligand-binding systems: applications to radioimmunoassay and plasma steroid-protein equilibria. Proceedings of Soc. Mex. Nutr. y Endocrinologia, Acupulco, Mex., Dec. 1970, pp. 615-632

Rodbard, D., Vaitukaitis, J., Ruder, H., Jacobs, H.: Mathematical analysis of kinetics of radioligand assays: improved sensitivity obtained by delayed addition of labeled ligand. J. Clin. Endocr., in press

Rodbard, D., and Lewald, J. E.: Computer analysis of radioligand assay and radioimmunoassay data. Research Methods in Reproductive Endocrinology. Acta Endocr. II, Copenhagen, Periodica, 1970, pp. 79-103

Mechanism of Action

Braunstein, G., Reichert, L., Van Hall, E., Vaitukaitis, J., and Ross, G. T.: The effects of desialylation on the biological and immunologic activity of human pituitary luteinizing hormone. Biochem. Biophys. Res. Com. 42: 962-967, 1971

Bridson, W. E., and Kohler, P. O.: Cortisol stimulation of growth hormone production by human pituitary tissue in culture. J. Clin. Endocr. 30: 538-540, 1970

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Kohler, P. O., and Bridson, W. E.: Isolation of hormone-producing clonal lines of human choriocarcinoma. J. Clin. Endocr., in press

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Vaitukaitis, J., Ross, G. T.: Altered biologic and immunologic activities of progressively desialylated human urinary FSH. J. Clin. Endocr., in press

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Van Hall, E. V., Vaitukaitis, J. L., Ross, G. T., Hickman, J. W., and Ashwell, G.: Effects of progressive desialylation on rate of disappearance of immunoreactive HCG from plasma in rats. Endocrinology, in press

Van Thiel, D., Bridson, W., and Kohler, P. O.: Induction of "Luteinization" in cultures of granulosa cells by chorionic gonadotropins. Endocrinology, in press

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Vanha-Perttula, T., Kohler, P. O., and Frohman, L. A.: Effect of insulin on growth hormone (GH) induction by cortisol in rat pituitary tumor cells. Life Sciences 9: 805-813, 1970

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Bermudez, J. A., Doerr, P., and Lipsett, M. B.: Measurement of pregnenolone in blood. Steroids 16: 505-515, 1970

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Slivka, J., Cargille, C. M., and Ross, G. T.: An analysis for superimposed biological rhythms: application to FSH and LH in women. J. Appl. Phys., in press

Strott, C. A., Bermudez, J. A., and Lipsett, M. B.: Blood levels and production rate of 17-hydroxypregnenolone in man. J. Clin. Invest. 49: 1999-2007, 1970

Vaitukaitis, J. L., Hammond, J., Ross, G. T., Hickman, J. W., and Ashwell, G.: A new method of labeling human chorionic gonadotropin for physiologic studies. J. Clin. Endocr. 32: 290-293, 1971

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Bardin, C. W., and Kirschner, M. A.: Clinical usefulness of testosterone measurements in virilizing syndromes in women. In Sunderman, F. W. and Sunderman, F. W., Jr. (Eds.): The Laboratory Diagnosis of Endocrine Disorders, Springfield, Ill., C. C. Thomas, in press

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Serial No. HD-RP1
1. Reproduction Research Branch
2. Section on Endocrinology
3. NIH, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Physiological and Pharmacological Studies on Reproductive Processes in Laboratory Primates

Principal Investigator: Dr. W. W. Tullner

Other Investigators: Dr. Gary Hodgen, Dr. Wilbert Nixon, Mr. Charles Turner, and Mr. Donald Barber

Man Years:

Total: 10
Professional: 4
Other: 6

Project Description:

Objectives:

1. To define the reproductive patterns of the rhesus monkey to facilitate experimental study.

2. To purify monkey gonadotropins and develop methods for their determination.

3. To examine the regulation of corpus luteum function in the monkey.

Methods Employed:

This project necessitates the maintenance of a primate facility, the capacity to work with monkeys, to characterize their menstrual cycles completely, and to breed them. Bioassays and radioligand assays for steroid and protein hormones are employed. The analysis of isoenzymes specific for certain tissues is necessary to quantify tissue-specific effects.

Major Findings:

In continuing our studies on the role of chorionic gonadotropin in early pregnancy in rhesus monkeys (Macaca mulatta) we have observed that treatment with human chorionic gonadotropin (50 to 500 IU/day) during the luteal phase of the menstrual cycle produced a marked rise in urinary estrone and plasma progesterone. Although injection of HCG prolonged the

cycle by ten days to two weeks, the early marked rise in plasma progesterone concentration could not be maintained. The alterations seen in progesterone levels mimic those reported for the implantation phase of gestation in this species. Concomitant with these observations, urinary estrogen (estrone) levels fluctuated between high and moderate levels at intervals of 2-4 days throughout the period of HCG treatment. In ovariectomized monkeys, HCG treatment had no effect on the pretreatment basal plasma progesterone levels indicating that increases noted were not influenced by adrenocortical progesterone secretion. These findings indicate that the ability of the corpus luteum to secrete progesterone under the influence of HCG is limited. This is in accord with an earlier report from this laboratory describing degenerative changes in the cells of the corpus luteum after the 21st day of pregnancy in rhesus monkeys. The failure of the corpus luteum to respond to continued elevated levels of exogenous HCG confirms results obtained in women last year in this Branch as well as our findings that rhesus serum chorionic gonadotropin levels during early pregnancy are maximum at a time when plasma concentrations of progesterone have markedly decreased.

Studies of immunology of the chorionic gonadotropins of higher primates were extended to include the baboon and chimpanzee. When extracts from urine of pregnant chimpanzee and baboons were introduced into a radioimmunoassay system for human chorionic gonadotropins, inhibition between chimpanzee urine extract was proportionate to inhibition by human chorionic gonadotropins at all levels tested. Interestingly, similar results were obtained when extracts of urine of non-pregnant chimpanzees were used for inhibition, suggesting that LH of the chimpanzee had antigenic determinants similar to those of chorionic gonadotropins from this species and the human.

The baboon pituitary extract had inhibition curves similar to those of human chorionic gonadotropins; however, the chorionic gonadotropins of this species showed only a cross-reaction inhibition curve with HCG. Thus, for the baboon as for the rhesus monkey, antigenic differences exist between pituitary LH and chorionic gonadotropin while in the chimpanzee the two gonadotropins were antigenically similar.

Purification of gonadotropins from kaolin-acetone concentrates of pregnant rhesus urine (days 18-25) by standard procedures of preparative polyacrylamide disc gel electrophoresis and elution into distilled water has permitted the isolation of two distinct bands of biologically active gonadotropic hormone. The specific activity of these isolated gonadotropins was 20-25 times higher than that of the original urine extract. This method yields a 40-50% recovery of gonadotropic activity. One of the isolated gonadotropic preparations has been used successfully as an antigen in rabbits to develop useable titers of antibody for establishing a radioimmunoassay for rhesus chorionic gonadotropin. This same isolated gonadotropin was an adequate reagent for iodinated tracer preparations.

Because the sensitivity of existing assays was not adequate for assaying very dilute preparations of highly purified isoenzymes of carbonic anhydrase, a radiotracer assay for carbonic anhydrase 50 times more sensitive was devised. The assay involves the catalytic conversion of $NaH^{14}CO_3$ to $^{14}CO_2$

and its recovery in hyamine hydroxide. Among the advantages of this assay method are its improved sensitivity, specificity and superior control over the conditions of the assay.

A testis-specific isoenzyme of carbonic anhydrase was isolated from normal rat testis parenchyma and testicular artery using Sephadex column chromatography and preparative polyacrylamide disc gel electrophoresis. Although organic solvent extraction was not deleterious to the testis-specific isoenzyme, the kinetic characteristics of one erythrocyte carbonic anhydrase were altered by standard ethanol-chloroform extraction methods.

Following exogenous estrogen and/or progesterone treatment of either immature rabbits or ovariectomized, mature guinea pigs, carbonic anhydrase isoenzymes were isolated from uterus and erythrocyte extracts using preparative polyacrylamide disc gel electrophoresis. A uterine-specific carbonic anhydrase appeared on the 3rd day following the cessation of progesterone injection of estrogen-primed rabbits. Uteri taken from guinea pigs 3 days after the final estrogen treatment contained a uterine-specific isoenzyme. Regulation of carbonic anhydrase activity and the appearance of uterine-specific isoenzymes was influenced primarily by progestational stimulation in the rabbit and estrogen in the guinea pig.

Because of reports from other laboratories showing homosexual behavior between male rats following p-chlorophenylalanine and Pargyline treatment, the dependency of this response on sexual maturity, testosterone and hypophysectomy was investigated. The results showed that prepubertal rats were not able to manifest homosexual excitation in response to drug treatment unless exogenous testosterone administration accompanied p-chlorophenylalanine and Pargyline. Mature rats which had been hypophysectomized did not demonstrate drug induced homosexual behavior even when exogenous testosterone therapy was used. The data showed that p-chlorophenylalanine and Pargyline induced homosexual activity between male rats was dependent on testosterone in the immature intact rat and on an intact pituitary in the sexually mature rat.

Significance to Bio-Medical Research and to the Institute:

The standardization of a model that is adequate for the study of ovarian function and pregnancy is key to the future development of human reproductive biology. There are many important questions that can be answered only by appropriate manipulation of an animal model and the small animals have been proved inadequate for this purpose.

Proposed Course:

Attempts to develop a radio-ligand assay for monkey FSH will be accelerated. The influence of hormones on early stages of spermatogenesis will utilize immature monkeys thereby creating the need for a more active breeding program. The hormonal events of early pregnancy and the role of chorionic gonadotropin will be examined in timed pregnancies. The use of specific testicular isoenzymes to characterize disorders of spermatogenesis will be started.

Honors and Awards: W. W. Tullner

Research Associate, Department of Zoology, Graduate School, Howard University, Washington, D. C. 1970-

Invited Lectures:

University of Massachusetts, Amherst, Massachusetts, November, 1970.

New Drug Seminar of the Chemotherapy Program of the National Cancer Institute, Bethesda, Maryland, November, 1970.

Armed Forces Institute of Pathology, Washington, D. C., April, 1970.

Publications:

Nixon, W. E., Tullner, W. W., Rayford, P. L. and Ross, G. T.: Similarity of antigenic determinants in pituitary and chorionic gonadotropins from primates. Endocrinology 88:702-706, 1971

Hodgen, G., Falk, R.: A radiotracer assay for carbonic anhydrase. Int. J. Appl. Radiat., in press

NICHD Annual Report
July 1, 1970 through June 30, 1971
Reproduction Research Branch
Reproduction Anthropometry Section

A Summary Statement

Reproductive capability occupies a limited span of time within human life. The research program of this Section centers in the origination, fruition and senescence of this life span within feminine life. We have shown this year that a woman's capacity to reproduce, as measured by the interval from menarche to menopause, has varied in our studies from as few as 22 years to as many as 45 years. This is but one of many facets of feminine reproductive function which is characterized by great variability.

Quantitative definition of variation patterns in menstrual cyclicity and gestational intervals, originally the objective of this research program, has required unexpectedly long-term persistence to complete. That end is now in view; this report covers the last year of assembly activities of the menstrual histories that are contemplated to form the data base for final reports under the program's present direction. We have been concerned with extension of the analyses to include many other variables of record. These involve duration of flow, gestational intervals for all products of pregnancy, the impact of oral contraceptives on menstrual cyclicity and fertility, secular trends in age at menarche and at menopause, the correlation in menstrual and gestational characteristics between mothers and their daughters, seasonal and incidental correlates of menstrual irregularity, and racio-ethnic differences in these reproductive functions.

These same years have also seen rapid development in the capabilities of the data processing machines upon which we must depend for analysis of such massive amounts of data. This has necessitated redesign of coding and concomitant revision of computer programming as we have striven to attain high levels of efficiency in all analytical operations to be performed by computers.

The effects of oral contraceptives on the menstrual cycle continue to be studied as we analyze the descriptive statistics of interval and flow for users of this method of pregnancy control. For nearly 1000 such persons we are in a position to relate measurements of typical value and variability before, during, and after use of these hormonal preparations. For most of them these findings are being related to their responses to a uniform set of questions concerning general health effects they have experienced while using the various pills.

Exploration of recognizable causes of abnormal genital bleeding has been the basis for a clinical program conducted with Eskimo women brought

from their villages to PHS Alaska Native Hospitals in Bethel and Barrow. The episodic nature of abnormal genital bleeding (AGB) has necessitated restriction of these examinations to a little over 30 AGB cases and a corresponding number of normal women matched for age, pregnancy experience, etc. As a stimulant to the interest of all Eskimo women in the 9 villages chosen for our menstruation and reproduction program in Alaska, Dr. Berman and an assisting Public Health Nurse have conducted an educational program in the physiological processes involved in human reproduction.

Our concern with the true period of human gestation, as distinguished from the interval from onset of last menstrual period (LMP) to birth, has been maintained this year. Our demonstration that recording the date of LMP in birth registration procedures removes ambiguity from the record is expected to remove a stumbling block to studies involving the "length of pregnancy". This is expected to have a helpful effect on the use of official birth statistics to attain public health goals. We have also undertaken another probing of the problem whether the observed racio-ethnic differences in gestational interval reflect a difference in basic biological processes. Some would ascribe the differences to chance correlates of socio-economic factors. In this we are working cooperatively with the Perinatal Research Branch of NINDS in analysis of their completed "obstetrical phase" data.

Much is due to the very large number (approximately 4,000) of women and their daughters who have maintained the habit of careful record keeping over the years covered by this program. Useful to them individually in personal planning, this activity has nevertheless involved a self-discipline not easily acquired and retained. We recognize these participants as dependable collaborators to whom untold credit belongs for allowing this program to reach its present maturity.

Serial No. HD-RP4(7)(c)
1. Reproduction Research Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Rhythmometry of Genital Bleeding as a Function of Human Reproduction

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigators: Michael L. Berman, M. D.
Ida L. Hellman, M.S.H.
Wayne Ewy, Ph.D. (University of Minnesota)
Kenneth Hanson, M.S. (AHRC, Alaska)

Cooperating Units: University of Minnesota Health Service and School of Public Health
Arctic Health Research Center, Alaska

Man Years:

Total: 2.8
Professional: 1.4
Other: 1.4

Project Description:

Objectives:

(a) To define the patterns of temporal variation in genital bleeding, from menarche to menopause, for five groups of participating women.

(b) To define any systematic differences between races of persons, and between communities of persons within the same race, in their patterns of genital bleeding, rhythmic and arhythmic.

Methods Employed:

Complete histories of genital bleedings, their interruptions by pregnancy, and modifications of those patterns (if any) associated with the use of drugs or mechanical devices to control pregnancy, have continued to be assembled from the sources established earlier in the development of this program. Those sources have been described in the report on this project for FY '69 and will not be repeated here.

Dated records of all genital bleedings, and medical information concerning related matters, are returned periodically to a receiving office in this program. Eskimo teenagers and women, comprising one of the 5 panels of participants, use monthly calendars which are gathered at times of interview by nurses and aides in the field approximately every 2 months. Edited and supplemented by notes at the time of interview, these records are returned to the Anchorage Field Station of AHRC (vide infra) for evaluative editing and coding before further processing of the coded information by staff of this Section.

Manual methods of statistical analysis of our data, used intensively in the past several years as a means of checking accuracy of the computer programming, have been reduced to a minor level during FY '71. This has been necessitated by the loss through death of a colleague (Dr. Behn) from joint direction of this program. Computer operations on the magnetic tape-file of data must now meet all our requirements; fortunately, those operations are proceeding with considerably increased efficiency. The addition of a full-time assistant director to the staff at the University of Minnesota conducting these computer operations under contract has contributed materially to this increase in output of analyses from that source.

Major Findings:

During FY '71 in response to repeated inquiry we have disclosed that the age at menarche in this country, as shown in our records, has deviated from the past downward secular trend which has been claiming a lot of public attention in recent time. A leveling-off of this trend occurs in our data for those born in the period from 1930 to 1950. Since then it is clear that an upswing has occurred in age at menarche for the newer generation. Our analyses have also revealed an unsuspected artifact of sampling which will require revision of the trends inferred by Tanner and others who have used averages for limited age-groups in conjunction with the year-of-occurrence scale of time for age at menarche.

The pattern of variation in age at menopause as given by our records has been presented recently before the North American Conference on Fertility and Sterility. Combined with the information on age at menarche as recalled over 30 years ago upon entry into this study by the 325 women who have met our rigid requirement for confirmed menopause, it has been possible to construct first approximations to expectancy curves for reproduction potential throughout menstrual life.

Our data resources are being analyzed currently in development of reports for publication on the following subjects:

(a) The proportion among all pregnancies of recognized abortions taken in relation to the gestational intervals.

(b) Reproduction capacity of the human female, from menarche to menopause.

(c) Secular trend in age at menarche; U.S.A. data for the 20th century.

(d) Menopausal irregularities in menstruation.

(e) Effects of oral contraceptives on the menstrual cycle.

(f) Racio-ethnic differences in gestational interval.

(g) Menstrual periodicity and fertility: The short cycle problem.

(h) Mother-daughter correlations in menstrual period characteristics.

Significance to Bio-Medical Research and the Program of the Institute:

When dependable and widely useful indicators of the imminence of ovulation become available, more natural methods of birth control may be used without the dangers attending artificial changes in bodily hormonal balances. In the absence of such indicators, much could be accomplished if fully acceptable means became available for reducing the intra-person variability of the interval between menstrual onsets. This project has gone a long way toward defining fully the extent and nature of that variability. Participants in the program of recording all genital bleedings are uniquely suitable as subjects for study in the exploration of causes of that variation, and in the development of innocuous agents for worthwhile reduction of that variation.

Proposed Course:

Definitive description of accumulated data has been published with respect to one variable only - the interval between menstrual onsets - and necessarily there in a preliminary way. The number of person-years of recorded experience which formed the basis of that initial report will be approximately doubled at the close of this fiscal year. At that time it is anticipated that a new analysis of the accumulated data, free of the previous restrictions involving age estimations, will be undertaken. Earlier deficiencies in the number of menarcheal and menopausal experiences will also be removed. A monographic report covering all aspects of operation of the project through its 4 decades of data assembly is projected.

In preparation for that final analysis, many computer programs have been completed and several others currently under development will be ready. These programs are all written in generalized form to permit easy adaptation to specific variables and their statistical analysis.

Major Findings:

Our earlier analyses of gestational interval for mothers classed as White, Negro and Puerto Rican in the NINDS/PRB program showed clearly that not only were Negro babies born about 6 days earlier than the White, with the Puerto Rican between these two, but also that the frequency distributions were of different character. Very short-term babies are delivered much more frequently in the non-white races, the distributions merging only in the extremely long-term cases. We consider the presumption that socio-economic differences are solely responsible for these differences in uterine developmental time is of debatable validity. Our variance analyses of the completed PRB data will segregate hospital and racio-ethnic effects independently, as previously. Since it is already well established that the socio-economic ratings of the mothers differ by hospital in the PRB data, we will be concerned to see to what extent removal of the hospital effects succeeds also in removal of socio-economic effects.

Significance to Bio-Medical Research and the Program of the Institute:

The current concept that a uniform rate of intra-uterine development of a product of pregnancy prevails for all gravida should be subjected to careful scrutiny. The considerable variation in post-partum rate of development of the newborn, which is so readily observable, may also be true for intra-uterine development. To assess the various intra-uterine growth factors with greater precision, it will be necessary first to find reliable criteria of the beginning in time of the pregnancy state. The occurrence of cyclic genital bleedings after initiation of pregnancy is accepted by some obstetricians as a fact with which we must contend in determining the true LMP date. If such bleedings can be differentiated in a practical way from true menstrual flows, much would be accomplished to remove the ambiguity involved in using the term "weeks of gestation" for the interval from last onset of genital bleeding to birth. The theoretical concept that maternal menstruation cannot occur after establishment of a pregnancy needs critical reconsideration based on practical issues.

Proposed Course:

It is intended to terminate this project when analysis of the PRB data is completed.

Publication:

Hammes, Laurel M., and Treloar, Alan E.: Gestational Interval from Vital Records. Am. J. Public Health 60: 1496-1505, 1970

Serial No. HD-RP5(7)(c)
1. Reproduction Research Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biometric Studies of the Human Gestation Period

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigators: William Weiss (NINDS)
Rudolph Vollman, M.D. (NINDS)

Cooperating Units: Arctic Health Center, Alaska
Office of Biometry and Perinatal Research Branch, NINDS

Man Years:

Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives:

(a) Analysis of the effects of data sources on the probability distribution of the interval from onset of last menstrual period (LMP) to birth (or delivery) for Caucasian pregnancies, using the precise data of the menstrual history program as the standard of reference.

(b) Study of racio-ethnic differences and hospital effects in the gestational interval using data from the completed obstetric phase of the NINDS/PRB research program.

Methods Employed:

This Section is privileged to be given access to the files of the Perinatal Research Branch, NINDS, for the purpose of bringing up-to-date an earlier study of racio-ethnic differences in the human gestational interval. Coverage of the completed "obstetrical phase" in this most unusual resource will terminate the updating procedures with which we have been concerned. Computer runs are currently being designed to yield the desired distributions.

Serial No. HD-RP6(9)(c)
1. Reproduction Research Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Effects of Oral Contraceptives on the Menstrual Cycle and on Health

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigators: Michael L. Berman, M.D.
Wayne Ewy, Ph.D. (University of Minnesota)

Man Years:

Total: 0.9
Professional: 0.6
Other: 0.3

Project Description:

Objectives:

(a) To evaluate the influence of oral contraceptives (OC) on the human menstrual cycle.

(b) To define the enduring effects, if any, of OC usage after cessation of treatment.

(c) To study the side-effects of these drugs on general health as it is perceived by the participants in the menstrual history program.

Methods Employed:

A medical report form is returned on an annual basis by every nonnative participant in the menstrual history study. This report has entries concerning pregnancy control drugs used during the year, name, dosage, schedule, and reason for discontinuing any regimen. Participants also volunteer additional information concerning health effects.

In an effort to secure responses to a uniform set of questions concerning health effects, a special reporting form mailed to all participants was returned by 90 per cent of the enrollees in the program. Coding of these responses in accord with a carefully drawn protocol has been transferred to magnetic tape. Tabulations of the response frequencies have been completed.

Descriptive statistics of menstrual intervals (means and standard deviations) have been calculated by desk methods for each OC user for the 3 (or 2) successive periods in her history (before, during, and after usage) that provided at least 4 consecutive menstrual periods. Approximately 1000 persons contributed data to this analysis for the calendar years prior to 1969. The intra-person correlations of these statistics have been studied numerically and graphically for the assessment of all observed differences. This study is currently being repeated by computer using all data now available on the master tape (i.e., inclusive of CY '69).

Major Findings:

The preliminary results arising from the special report on personal reactions to OC usage as given last year have not been altered by the consistency testing against previously recorded history. The volume of special correspondence on PCD usage by study participants incites conjecture that adverse publicity since that time (Fall, 1968) has led to an increase in rejection of the oral contraceptives as a birth control measure. This has not been tested, however, by computer analyses for change in the numbers of oral contraceptive and IUD users.

The analysis of descriptive statistics assembled through CY '68 differentiates oral contraceptives into the combination (COC) and sequential (SOC) types, ignoring the changes in strength of these types as sold commercially during the period covered. Users of OC did not differ in the statistical characteristics of their collective menstrual cycles prior to adopting either type of pregnancy control. COC users averaged slightly shorter menstrual cycles than SOC users experienced, but the latter were somewhat more regular in their controlled cycles (-this may be influenced by lesser adaptability of SOC to manipulation of the onset date). COC users experienced some lessening of the flow period; this is not apparent with SOC. Failures of occurrence of withdrawal bleeding were more frequent with COC. Those who changed from COC to SOC became more stable in both cycle length and duration of flow. Termination of oral contraceptive use, regardless of type, was marked in general by a return to the pre-OC type of menstrual experience, but considerable variation among persons exists in this. Certainly the OC experience did not serve in general to be a regularizer after it was dropped.

Significance to Bio-Medical Research:

A problem of major importance in evaluation of the reaction to pregnancy control measures is that of securing a population at risk which is not self-selected in such a way as to contribute substantial bias to the results. While the participants in this study of cyclicity in a basic reproductive function were self-selected, the matter of pregnancy control by artificial means did not enter the self-selection process unless it kept a user out of the study. Thus this population can be studied to better advantage without risk of prejudice than alternative populations becoming available for study because of their desire for pregnancy control or need for medical supervision.

Proposed Course:

The study of reactions, psychological as well as medical, to contraceptive drugs and mechanical devices will be maintained in this rather uniquely suitable population group for at least another year. As opportunity presents itself, or is specifically provided by design, to increase or introduce professional clinical observation of the individuals involved in this study, it is desired that maximum advantage be taken of that opportunity.

Serial No. HD-RAS1(c)
1. Reproduction Research Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Endemiology of Abnormal Genital Bleeding Among Alaskan Eskimo Women

Principal Investigator: Michael L. Berman, M. D.

Other Investigators: Alan E. Treloar, Ph.D.
Mortimer B. Lipsett, M.D.
Richard A. Malmgren, M.D. (NCI)
Ralph D. Powell, Jr., M.D. (NCI)

Cooperating Unit: Pathologic Anatomy Branch, NCI

Man Years:

Total: 1.3
Professional: 1.1
Other: 0.2

Project Description:

Objectives:

(a) To determine statistically the scope of non-cyclic genital bleeding among this race of indigenous people, and

(b) To assess clinically the proportion of cases attributable to recognized casual factors.

Methods Employed:

Menstrual histories have been recorded by nearly all Eskimo women in nine villages, and by self-selection samples of these indigenous peoples in the towns of Bethel and Barrow, for periods of as much as three years and eight months. Approximately 800 persons are enrolled in this activity. Analyses of these records in terms of definitions of "normal", "abnormal", and "indeterminate" genital bleedings established for the total program of menstrual histories are in progress to determine the proportions of these types of genital bleedings among Eskimo women in Alaska. Equivalent analyses are being made for Caucasian women living elsewhere as well as a small group resident in Anchorage and its environs.

Critical study of individual histories has enabled selection of a representative group of cases of AGB among Eskimo women for thorough clinical study of each case on a voluntary basis. This includes a complete pelvic examination in addition to a general physical work-up. Tissue examinations will be completed by staff of the Pathologic Anatomy Branch of the National Cancer Institute, and hormone assays in blood plasma and urine samples by the Branch.

Major Findings:

In a review of an unbiased sample of 347 Eskimo records of all genital bleedings, we found 17 per cent of the case histories indicated abnormality. The corresponding figure for over 1500 Caucasian women is slightly less than 5 per cent. Likewise a small group of Caucasian women in Alaska shows 5 per cent of AGB. This difference is unquestionably significant in the statistical sense.

From desk studies of sample data we fail to detect any indication that length of day and its seasonal correlates have any relationship to the incidence of abnormal genital bleeding. This conclusion is supported by lack of differentiation of the records from Barrow in the Arctic from those assembled in the sub-Arctic region around Bethel.

All clinical examinations will be completed before the close of FY '71. No attempt to analyze the data for differences is being made until all clinical results are available.

Significance to Bio-Medical Research:

The differentiation of menstrual bleeding as a reproductive function from other genital bleedings has become a major problem in the research program of this Section. We seek in this project to take essential first steps in determining the extent to which recognized pathological conditions can appropriately be considered causal to AGB. Eskimo women provide a very suitable population for such inquiry not only because of the heightened prevalence of the AGB condition, but also because of (a) their highly cooperative attitude toward research in the basic biology of reproduction functions, and (b) their availability on a purely voluntary basis for clinical procedures in nearby Alaska Native Hospitals.

The problem of differentiation of "menstrual flows" from other genital bleeding is simple only in terms of speculations based on hormonal control theory. We believe we have shown clearly that conventional ideas concerning menstrual cyclicity are not attuned to the facts. Differentiation of a "menstrual" flow from other genital bleedings by reliable technical procedures is a goal still to be achieved. We seek to avoid attributing unexpected genital bleedings to pathological conditions until reasonable proof of this is available.

Proposed Course:

This investigation will be closed with FY '71 as far as field work by staff and GB record-keeping by Eskimo women is concerned. It is anticipated that statistical analysis of all the data assembled during the field operations will be completed in FY '72.

NICHD Annual Report
July 1, 1970 through June 30, 1971

Reproduction Anthropometry Section
Collaborative Research Reimbursable Agreement Report

Contract Title: Statistical Studies of Reproduction among Alaskan Natives

Contractor: Arctic Health Research Center (PHS), Alaska, on behalf of its Epidemiology Section, Anchorage Field Station

Money Allocated: $59,100

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigators: Mrs. L. M. Hammes, B. A.
M. L. Berman, M. D.
Mrs. Carol Baum, B. A.

Contract Description:

Objectives:

(a) To gather currently recorded histories of genital bleeding and reproduction from participating Eskimos in the Yukon-Kuskokwim delta area and in Barrow.

(b) To edit those histories, code them in accord with protocol, and otherwise process and file the basic records received.

(c) To provide the administrative services necessary for conduct of clinical field investigations for a Clinical Associate from NICHD staff assigned to duty in Alaska in connection with the NICHD investigation of reproductive functions.

Major Findings:

Monthly calendars for recording of all genital bleedings by the participating Eskimo women and girls have been collected on periodic visits to homes in native villages and the towns of Bethel and Barrow by or under the supervision of field nursing staff of AHRC. The field staffing problems which led to losses of basic data from the Eskimo women in 1969 were rectified with the addition of Dr. Berman from our staff to exercise supervisory functions in Alaska. Editing and coding of the data received from the field has proceeded in Anchorage throughout the year according to schedule. Completion of this activity must be transferred to Section Staff in Bethesda with cessation of the contract support at the close of FY '71. Statistical analyses of all data from the Eskimo panel will then proceed as one component of the total assembly of data on menstruation and reproduction history.

Significance to the Research Program of this Institute:

The four panels of Caucasian record-keepers are self-selected as participants and as such do not constitute random samples of their communities. If communities of widely divergent character and environment do not differ appreciably in the cyclic pattern of their menstrual phenomena, then this self-selection feature of the participants ceases to be critical in many aspects of representativeness issues. We believe we have been remarkably fortunate in securing this panel of record-keepers from among the Alaskan Eskimo on this account, particularly since nearly 100 per cent of menstruating women in the selected villages agreed to keep the desired records.

In addition to race, many factors such as social organization, manner of living, climatic environment and length of day, differentiate between the Eskimo and our Caucasian panels as communities of participants. Therefore we do not seek to assign any differentiation we may find between Caucasian and Eskimo in these studies to race alone. A comparison of the Eskimo and Anchorage-Caucasian panel experiences will bear more directly on race and social organization since the differences in climate in this comparison are rather minor.

It remains possible that separate factors independently may have differentiation capacity as causal forces, yet so interact or confound one another that real differentiations between panels will vanish when all factors are combined. Our inferences on the importance of race in the feature of biology under investigation must necessarily be very guarded. Many racial and environmental contrasts must be studied before generalizations to specific factors will assume that reasonableness acceptable as proof. The Alaskan Eskimo series is therefore regarded as an initial probing into a very complex field of inquiry.

Dependable findings will not be available from the Alaskan segments of our data until the results of statistical processing become available. It is stimulating, however, to note from visual inspection and preliminary manual operations on parts of the Alaskan data that any differences in the general pattern of intervals between menstrual onsets are too small to attract notice at this stage of the inquiry. On the other hand, there is stronger evidence that the durations of genital bleedings are substantially longer among the Eskimo.

NICHD Annual Report
July 1, 1970 through June 30, 1971

Reproduction Anthropometry Section
Collaborative Research Contract Report

Contract Title: Quantitative Definition of the Temporal Characteristics of the Human Menstrual Cycle

Contractor: University of Minnesota, on behalf of its Biometry Department, School of Public Health

Money Allocated: $40,298

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigator: J. E. Bearman, Ph.D.

Contract Description:

Objectives:

(a) To provide data processing and statistical analysis services via computer operations for the studies of genital bleeding of the human female as a reproductive function.

Major Findings:

Through FY '71 the University Health Service has continued to contribute office space and equipment, the Biometry Department of the School of Public Health has provided directional services and ancillary facilities for computer operations, and the Medical School has cooperated in providing full access to all its computer equipment. Working closely with staff of this NICHD Section, the University (a) has completed the revision of its processing techniques, (b) has updated the master file on magnetic tape to include all data from the original and new Minnesota cohorts and their daughters for the calendar year 1969, (c) has created a master tape for all data from the Alaskan Eskimo series for a 26 month period, (d) has added to the magnetic tape files all coded data from the special report on pregnancy control drug usage, (e) has supplied the Section with many print-outs as requested, and (f) has continued its writing and "debugging" of programs for the production of information being sought by the Section.

The appointment of Dr. Ewy as a full-time assistant director for the contract operations for the last 4 1/2 months of this fiscal year has proven a most important step in strengthening operational contacts between the Section and contract staffs. An increased output of analytical operations - as opposed to input processing - has been made possible for the last period of this fiscal year by this appointment.

Significance to the Program of the Institute:

The current research program of the Institute concerning genital bleeding was initiated 38 years ago. The data-assembly operations for the three "Minnesota panels" in the program have been conducted continuously since then in the name of the University. Through its Health Service Director, and the writer, the University shares in the guarantee of confidentiality of the records provided by each participant. Thus the University, through this contract, maintains an active interest also in the data processing operations. These are of long duration in this field and have matured into a very valuable specialist capability. It has proven very much in the interest of the Institute to have the University serve thus as one of its operating arms in prosecution of this research program.

Proposed Course:

The input of menstruation and reproduction data to the magnetic tapes from the 5 panels of participants closes with the current fiscal year. The final statistical analysis and interpretation operations for the total program by the current principal investigator is planned to commence in the following year. The writer anticipates that this contract with the University of Minnesota will be relied upon to produce not only the necessary computer output, but also statistical assistance in all matters of detail for the preparation of reports. Concurrently, investigation will be undertaken of the possibility of classifying genital bleedings into meaningful types by computer programing. This latter endeavor is currently in preparatory stages of development; if successful it will not only remove a most troublesome problem for coders, it should also contribute materially to resolution of definitional problems surrounding menstrual versus "non-menstrual" bleedings.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Social and Behavioral Sciences Branch

Summary of Research Activities

The Social and Behavioral Sciences Branch has been engaged in a number of investigations in which applied and theoretical interests converge. The focus of the research program is on early environmental influences on cognitive and personality development. Theoretical issues concerning early experiences have a special relevance at the present time as programs are being developed to overcome and mitigate the effects of extreme deprivation on young children growing up in impoverished environments.

Several studies have been centered on problems of early environmental influences on cognitive and personality development. A major goal has been to develop techniques for studying mother-infant interactions in the home, and teacher-child interactions in preschool settings. We have conceptualized environmental variables in terms of several theoretical orientations, e.g., Piagetian developmental theory, social learning theory, and have then translated these theoretical concepts into observation categories.

One study has been concerned with analyzing the environment of six month old infants from lower socioeconomic backgrounds, and studying relationships between patterns of early stimulation and the development of cognitive and personal-social functions. The findings emphasize the value of differentiating the dimensions of the infant's environment, and suggest that it may not be very meaningful to label environments grossly as depriving. We find relatively low relationships between dimensions of the inanimate and social environments. We also find that specific dimensions of the early environment are significantly related to specific aspects of infant functioning. For example, level of social stimulation relates significantly to measures of the infant's social responsiveness and early vocalization; properties of inanimate stimulation, particularly variety, were more strongly related to the Bayley Psychomotor Development Index and to measures of exploratory behavior and preference for novel stimuli. Measures of cognitive-motivational functions, which index the infant's persistence and active attempts to reach out and have an effect on the environment, are significantly related to both characteristics of the inanimate and the social environment. The major findings of this study were reported in a symposium, consisting of four papers, at the meetings of the Society for Research in Child Development. Chapters have been drafted which will form the nucleus of a monograph.

A follow-up investigation of the infants and mothers studied at six months has been completed. The purpose of this study is to analyze the significance of the early environment for development at 18 months, and to describe the patterns of mother-child interaction in this social group at a time when a central issue for the child is the development of autonomy. A series of structured situations were developed to analyze the ways in which the mother handles the child's exploratory behavior, her response to the child's

autonomous and dependent behavior, and her techniques of teaching the child some simple problem-solving tasks. Coding and some statistical analyses have been completed. As a group, these children have shown a general decline in intellectual functioning, as measured by the Bayley Test of Infant Development.

We have completed data analyses in a study of the effects of experiences in a Headstart program on the personality and cognitive development of children from "disadvantaged" backgrounds. In general, there were few significant relationships between cognitive input and changes in cognitive functions. Significant relationships were found between the teachers affective interaction with the children--the extent to which she identified and labelled feelings--and changes in the children towards a more positive self-concept. A time-sampling observational procedure was developed to enable a finely differentiated analysis of the preschool environment--the varieties of cognitive stimulation, techniques of teacher control, and the kinds of reinforcement and evaluative feedback given by teachers. A report on the observation instrument will be presented on a symposium at the annual meetings of the American Psychological Association in September, 1971.

There exists considerable controversy regarding the relative merits of various types of preschool intervention programs. Resolution of these controversies is hampered by the lack of detailed observational data on the characteristics of these programs. We have just completed a study directed towards refining the observational instrument developed for analyzing preschool environments. Two model programs having important differences in educational philosophy--a Montessori program and a Bank Street program--have been studied. The basic aim is to determine how effectively the observation categories discriminate between the two programs. The observational technique used in this study should provide a useful tool for studying various kinds of intervention programs.

One aspect of early environmental influence which has been essentially neglected is the impact of the father. With cultural changes in the degree of father's participation in child care, and with growing numbers of one-parent families, the need for better understanding of the role of the father becomes increasingly important. Theory and studies of father-absence suggest that the presence or absence of the father may have significant effects on sex-role identification, and on relative strength of cognitive abilities, e.g., quantitative vs. verbal. There are very few direct studies of fathers interacting with their children. Currently in a pilot stage is an investigation aimed at obtaining descriptive data on the father-child relationship. Work is underway on developing coding categories for analyzing videotape records of the family meal and parent-child interaction in a play situation. Comparisons will be made between the behavior of mothers and fathers in comparable situations with their 2½ year old first born children. A major goal is to develop a model for conceptualizing whole family interaction--the father-mother-child triad, and the patterns of reciprocal influences.

Until very recently most research on early environmental influences has been designed in terms of a unidirectional model, i.e., the influence of parental

behavior on the child. We are initiating studies in which child-environment interactions are conceptualized as a reciprocal feedback system. Several basic assumptions underlie this approach: 1) the young child is an active organism who shapes his environment; 2) the impact of any environmental event is moderated by the child's particular sensitivities and vulnerabilities; 3) the behavior which the child elicits from parents and other significant persons are, in part determined by his individual characteristics, e.g., activity level, irritability, social initiative. A first step in investigating this problem has been the development of techniques for assessing these characteristics in young infants and studying their consistency during the first six months. Pilot studies with four week old infants have begun.

Analyses have been completed of data from a longitudinal study of adopted children through a contract with Family and Child Services of Washington, D. C. A paper was presented at the meetings of the American Orthopsychiatric Association on one aspect of the findings, the relations between mothering experiences in infancy and cognitive and personality development at ten years. Several dimensions of maternal care were significantly related to IQ and to the child's capacity to establish discriminating relationships at ten years of age. These relationships were significant only for boys. An environment in infancy characterized by warmth, responsiveness to the child's cues, and one in which stimulation is sensitively adapted to the child's developmental level, is favorable for cognitive and emotional development of boys.

Further analyses of data from a study carried out under the auspices of the Group Health Association of Washington, D. C. on psychological adaptation of women to a first pregnancy and their early postnatal adaptation resulted in two reports. One, "Seven Women: A Prospective Study of Postpartum Psychiatric Disorders," presented at the Third International Congress on Psychosomatic Medicine in Obstetrics and Gynecology, consisted of an intensive analysis of the prenatal and postnatal adaptation of women who developed psychiatric disorders during the first six postpartum months. It was found that the occurrence and type of mental disturbance could, in certain cases, be predicted from the prenatal evaluations, but response to the delivery could not be predicted. Most of the women who developed mental disturbances in the postpartum period had precipitating stresses other than childbirth.

The results of other analyses of these data were described in a paper, "The Relationship of Antenatal and Perinatal Psychological Variables to the Use of Drugs in Labor." This paper was concerned with some of the determinants of the amounts of sedative and analgesic medication administered during labor. It was found that the amounts of these drugs administered was significantly related to prenatal measures of psychological adaptation. The use of these drugs was not related to psychological variables measured during labor or to other perinatal variables.

Professional activities of the staff have included presentations of research and theoretical papers at scientific meetings and special conferences. Frank Pedersen and Leon Yarrow participated in the program of the American Psychological Association. Leon Yarrow presented a paper on a symposium at the American Orthopsychiatric Association meeting. Papers were presented on

a symposium at the meetings of the Society for Research in Child Development by Leon Yarrow, Judith Rubenstein and Frank Pedersen. Walter Brown presented a report at the Third International Congress on Psychosomatic Medicine in Obstetrics and Gynecology in London. Joan Bissell gave a paper at the meeting of the National Association for the Education of Young Children, and a report on "The Cognitive Effects of Preschool Programs for Disadvantaged Children" at a conference on preschool education at the Harvard Graduate School of Education.

Leon Yarrow and Joan Bissell have served as consultants to the Office of Child Development. Joan Bissell has just completed an integrative review and summary of the report on the implementation of planned variation in Headstart for the Office of Child Development.

Leon Yarrow has served as president-elect and as a member of the Governing Council of the Society for Research in Child Development, and as consulting editor of the Merrill Palmer Quarterly of Human Development, and on the editorial board of the Monographs of the Society for Research in Child Development.

Dr. Joan Bissell, a developmental psychologist, joined the staff in July 1970 as a Staff Fellow. Dr. William Davidson, a psychiatrist, became a member of the staff as a Research Associate in July 1970. Dr. Salvatore Lomonaco and Dr. David Fox completed their assignments as Research Associates in June 1970.

Publications

Brown, Walter A. and Mueller, P.S. Psychological function in individuals with amyotrophic lateral sclerosis. Psychosomatic Medicine, 1970, 32, 141-152.

Brown, Walter A. The meaning of success in an individual with a "success phobia." Psychiatry, 1971, in press.

Brown, Walter A., Kromberg, C. and Slavinsky, A. Staff attitudes towards research. Journal of Psychiatric Nursing and Mental Health Sciences, March-April 1971.

Burnes, Kay. Pattern of WISC scores for children of two socioeconomic classes and races. Child Development, 1970, 41, 493-499.

Klein, Robert P. Acoustic analysis of the acquisition of acceptable (r) in American English. Child Development, 1970, in press.

Yarrow, Leon J. Ethology, control systems, psychoanalysis and mother love. Contemporary Psychology, 1970, 15, 493-494.

Yarrow, Leon J. Maternal deprivation. In Encyclopedia of education. New York: Crowell Collier and Macmillan, Inc., 1971.

Smith, M.S. and Bissell, Joan S. Report analysis: the impact of Head Start. Harvard Educational Review, 1970, 40, 51-104.

Serial No. HD-GD4

1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Early Environmental Influences on Cognitive and Personality Development in Infancy

Previous Serial Number: Same

Principal Investigators: Leon J. Yarrow, Ph. D., Judith Rubenstein, Ph. D.
Frank A. Pedersen, Ph. D.

Other Investigators: Myrna Fivel, M.A.

Cooperating Units: D.C. Department of Public Health, Children's Hospital of the District of Columbia, Group Health Association of Washington, D. C.

Man Years:

Total:	1.75
Professional:	1.25
Other:	.50

Project Description:

Objectives: In this investigation of early environmental influences on cognitive and personality development during the first year of life, one major goal has been to develop an observational instrument to measure differentiated aspects of the early environment. A second objective was to study relationships between specific parameters of the environment and specific aspects of development during the first six months.

Methods Employed: The main sample, composed of 55 infants and their caretakers from low socio-economic backgrounds, were obtained through two well-baby clinics in the District of Columbia. To extend the range of environmental variables and to permit some exploration of the effects of social class status on early maternal care, this group was augmented by a sample of 15 infants from middle-class backgrounds obtained through a private medical care program. Pediatric and neurological examinations were conducted to exclude infants with abnormalities that might be associated with developmental retardation.

Through time-sampling observations in the home when the infants were five months of age, data on environmental variables were obtained. Data on infant characteristics have been obtained by means of the Bayley Scales of Infant Development, tests of selected cognitive functions, a measure of exploratory behavior and measures of social responsiveness.

Major Findings: Analysis of data has involved a number of steps: conceptual grouping of the time-sampling variables, obtaining basic frequencies on each of the environmental variables and combinations of variables; obtaining measures of reliability; and analyzing the interrelationships among the independent variables. Similar steps have been carried out in analyses of the dependent variables: conceptual ordering; reliability determination and analysis of interrelationships. Following these steps, univariate correlational analyses were carried out between the independent and dependent variables.

On the basis of conceptual considerations and statistical criteria, the number of independent variables was reduced to eight: three dimensions of the inanimate environment: variety of objects available to the infant, complexity of these objects and responsiveness or feedback properties; five dimensions of social stimulation: level, variety, expression of positive affect, contingent response to positive vocalization, and contingent response to distress. The social and inanimate stimulation dimensions are statistically independent of each other, supporting the view that global characterizations of environments as depriving or stimulating tend to be gross oversimplifications.

The major univariate statistical analyses have been completed. From the many significant relationships which emerged, several generalizations can be made. It was found that social stimulation variables are significantly related to vocalization rates and social responsiveness, while aspects of the inanimate environment are related to exploratory behavior and preference for novel objects. Goal directed behaviors, such as goal orientation, reaching and grasping, and secondary circular reaction, were related to both the social and inanimate environmental variables.

Many specific relationships of theoretical interest have emerged. For example, the frequency of caretaker's contingent vocal responses to the infant's vocalizations is significantly correlated with an independent measure of infant vocalization rate; whereas noncontingent vocal stimulation was unrelated to this measure. These results are consistent with an operant learning model and extend laboratory findings to the natural environment. The availability of toys which are highly responsive to the infant's manipulative efforts is significantly related to the development of secondary circular reactions, a stage of sensorimotor intelligence thought to be a precursor of problem-solving.

Multivariate analyses are being planned to determine whether complex interactions exist among the stimulation variables which may operate to accentuate specific stimulation or deprivation effects.

Four research papers presenting the detailed statistical results and the conceptual framework of the study were delivered at the biannual meeting of the Society for Research in Child Development, in Minneapolis. Chapters of a report in monograph form have also been drafted.

Significance to Biomedical Research and the Program of the Institute: In order to attain more precise understanding of environmental factors which facilitate and those which hinder the personal-social and intellectual development of the child, we need information on a broad range of patterns of maternal care in early childhood. Most of our generalizations about the effects of early maternal care are based on middle class samples or extremely depriving environments. In addition to the potential significance of the findings of this research for theories of early development, we expect that the techniques developed for home observations will be useful contributions to methodology.

Proposed Course of Project: It is planned to complete the multivariate analyses of the data, and report the major findings in monograph or book form.

Honors and Awards: None

Serial No. HD-GD3

1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effects of Preschool Experiences on the Intellectual and Personality Development of Children from Culturally Deprived Backgrounds.

Previous Serial Number: Same

Principal Investigator: Leon J. Yarrow, Ph. D.

Other Investigators: Robert P. Klein, Ph. D., Frank A. Pedersen, Ph. D., Kay Burnes, Ph. D., Sandra Sklar, M.A., Bette Marcus, M.A.

Cooperating Units: Office of Economic Opportunity, Project Headstart, National Capital Day Care Association

Man Years:

Total:	3.0
Professional:	2.5
Other:	.5

Project Description:

Objectives: This study has been concerned with the impact of early preschool learning experiences on the development of cognitive skills, behavioral controls and self-concept in four-to-five-year-old children from culturally disadvantaged backgrounds. A major goal has been to develop methods for differentiated analyses of preschool learning environments, utilizing concepts from social learning theory, cognitive developmental theories and operant learning theory. We have also been interested in studying relationships between cognitive functioning and behavioral controls in preschool children.

Methods Employed: The sample consists of 58 children in 12 preschool classrooms in Headstart and day care programs. Time-sampling observational techniques were used to analyze the preschool environment. To provide a sample of different classroom activities and minimize effects of day-to-day changes, each child was observed for 20 observational units on each of three days in a balanced order. These observations were made on each child at three different points in the school year--in the fall, shortly after the

beginning of school; in the middle of the term; and in the early summer, toward the end of the term. A comprehensive observational system was developed. This system is organized in terms of the following major categories: teacher behavior, which include dimensions of teacher control, cognitive input dimensions, the affective qualities of the interaction, and evaluative feedback; child-behavior variables, focusing on self-initiated or self-directed activities; and background and setting variables.

Measures of the dependent variables consisted of several standard tests and others developed especially for this investigation. For studying cognitive functions, the following techniques were used: the Wechsler Preschool Scale of Intelligence, several measures of language functions, the Johns Hopkins Perceptual Test, selected items from the Frostig Developmental Test of Visual Perception, and a specially designed object categorization test for assessing concept development. As measures of "behavioral controls," we have adapted the Maccoby motor inhibition measures, and have developed techniques to assess capacity to delay gratification, a measure of focused attention, and a situational test of response to a difficult task. In addition, we have developed a self-concept test designed to tap various aspects of the child's feelings about himself, his competencies and deficiencies, and his perception of how he is evaluated by other people.

Major Findings: Data collection and a major share of data analyses have been completed. Frequency counts for the classroom observational data have been carried out. Various aspects of teacher behavior and child activity in the classroom have been combined to yield scores on approximately 15 dimensions of classroom experience, e.g., teacher verbal cognitive input, teacher level of restrictiveness, prosocial controls. The mean frequencies of many of these dimensions are quite low. For example,the mean amount of cognitive input per child is about one-half hour a week of cognitive input from the teacher. Praise or some positive recognition of the child's behavior is also infrequent.

These findings on the cognitive and affective variables may be pertinent in interpreting the analyses of relationships between the child's classroom experiences and his cognitive and personal-social development. The relationships were low and, for the most part, statistically insignificant. Despite the restricted range in socioeconomic status of this low income group we found this variable a more important determinant of cognitive growth than the child's classroom experiences. Several multiple regression analyses were then carried out to test the relationships between amount of cognitive activities and cognitive skills, controlling for socioeconomic status. These analyses indicated that total amount of teacher cognitive input related significantly to verbal I.Q., and total amount of child cognitive activities related to performance I.Q. We are now analyzing the relationships between specific cognitive experiences and specific cognitive skills, controlling for socioeconomic status.

Another set of analyses has been concerned with the relationships between cognitive functions and behavioral controls. We did not find coherent clusters of behavioral control measures; nor were there significant relationships between cognitive functioning and behavioral controls.

A measure of self-concept developed for this study has shown a satisfactory reliability and also a reasonable degree of stability over a six month period. Some aspects of self-concept are significantly related to I.Q. and socioeconomic status. Significant relationships were also found between one aspect of the teacher's affective interaction with the children, the extent to which she identified and labelled feelings, and changes in the children toward a more positive self-concept.

Significance to Bio-medical Research and the Program of the Institute: Although there has been much research on basic learning processes in restricted experimental situations, the findings and concepts have had limited generalizability to more complex learning settings. Current controversy about the relative merits of various approaches to preschool programs emphasizes the need for greater conceptual clarity about the preschool environment. The concepts and techniques developed in this research should contribute to more adequate methodologies for studying complex environmental inputs in relation to the cognitive and personal-social development of preschool children. The substantive findings should have implications for the development of preschool programs.

Proposed Course of Project: Further multiple-regression analyses will be carried out. A report on the observation instrument is to be presented at the annual meeting of the American Psychological Association, September, 1971. A report on the self-concept instrument and on the major findings will be prepared.

Honors and Awards: None

Serial No. HD-GD6

1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Patterns of Maternal Behavior at Eighteen Months and the Personal-Social and Intellectual Development of Children from Culturally Disadvantaged Backgrounds

Previous Serial Number: Same

Principal Investigator: Leon J. Yarrow, Ph. D.

Other Investigators: Frank A. Pedersen, Ph. D., Kay Burnes, Ph. D., Robert Klein, Ph. D., Myrna Fivel, M.A. Salvatore Lomonaco, M.D.

Cooperating Units: None

Man Years:

Total:	1.75
Professional:	1.25
Other:	.50

Project Description:

Objectives: This study is the second phase of a longitudinal investigation of environmental influences on the development of children from low socioeconomic backgrounds. The major objectives are: (1) to analyze relationships between environmental influences at six months and cognitive and personality development at eighteen months; and (2) to study patterns of mother-child interaction of mothers from low socioeconomic backgrounds and to assess their impact on functions which mediate cognitive development, e.g., exploratory behavior, focused attention.

Methods Employed: The sample consists of 26 children from lower socioeconomic backgrounds and 12 children from middle class backgrounds. These children were first studied when they were six months of age. The major methods developed consist of a series of situational tests designed to sample the mother's techniques in handling the child's exploratory behavior, her approach in teaching the child simple problem-solving tasks, and in eliciting and maintaining his attention on a verbal task. The child's behavior has been studied through several situational techniques designed to investigate his spontaneous exploratory behavior with interesting toys, his initial response

to a stranger, his response to being separated from his mother, and his reaction to being deprived of his mother's full attention. Intellectual development was assessed by the Bayley Developmental Scales. Additional data on family social history and characteristic caretaking patterns were obtained through interviews with the mother.

Major Findings: Collection of data has been completed and analyses are in progress. Coding is almost completed of the exploratory behavior and the maternal teaching situations. The teaching codes have attempted to describe interactional patterns, i.e., the mother's response to the child's behaviors, e.g., restrictive feedback, corrective feedback, positive and negative evaluation of the child. The exploratory behavior codes index varying degrees of encouragement or restrictiveness of exploratory behavior, e.g., from simple attentiveness to active encouragement, from ignoring to facilitating the child's manipulation and exploration of the environment. In another situation, in which the mother is occupied, the codes focus on the child's autonomous and dependent behavior.

Comparison of the Bayley Mental Development Index at 6 and 19 months shows a significant drop on this measure of intellectual functioning. Correlational analyses show low individual consistency on this developmental index. Other analyses on the effects of continuity of caretaking in infancy indicate that lower class boys with a relatively stable caretaking history show more avoidance of strangers and less protest on brief separation from their mothers than children with a high discontinuity history.

Significance to Bio-Medical Research and the Program of the Institute: Current theories have emphasized the importance of early experiences for cognitive and personal-social development, and suggest that intervention must occur very early in order to be effective in preventing intellectual or personality damage. In developing programs to combat early psychosocial deprivation, it is essential that we identify more clearly the significant environmental variables, the developmental periods at which they may have a most significant impact, and the mechanisms through which they operate. This study should add to our understanding of some of the specific factors in the early environment which affect cognitive development.

Proposed Course of Project: It is planned to complete the coding and data analyses during the next year. A report on the relationships between continuity of caretaking in early childhood and response to strangers and separation reactions will be completed for publication.

Honors and Awards: None

Serial No. HD-SB1(c)
1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: An Observational Approach to the Measurement of Preschool Environments

Previous Serial Number:

Principal Investigators: Leon J. Yarrow, Ph. D., Joan Bissell, Ed.D.

Other Investigators: Sandra Sklar, M.A.; Alice Abramson, M.A.

Cooperating Units: Montessori Society of Chevy Chase, Bank Street College of Education

Man Years:

Total:	2.0
Professional:	1.75
Other:	.25

Project Description:

Objectives: This research is concerned with refinement and further development of a time-sampling observational technique for describing children's experiences in preschool settings. The observational technique was developed in an earlier study to assess the impact of experiences in Headstart programs. In this study two model preschool programs with important differences in philosophy are being compared. We are interested in determining whether the observational instrument can distinguish unique characteristics of different kinds of preschool programs. A second and related purpose of the study is to describe differences in children's experiences in programs based on different philosophies. There has been a proliferation of models for preschool intervention during the last several years, but there is as yet no observational research describing the ways in which these programs vary in terms of children's experiences.

Methods Employed: The sample consisted of 48 children in six preschool classrooms, three based on the Montessori 'prepared environment' model and three on the Bank Street College 'child-centered' model. To provide a representative sample of the different classroom experiences of children in the two models, each child was observed for ten observational units of two

minutes duration on each of six days. Our previously developed observational technique was refined for use in this study. The observational scheme describes the following dimensions: child-behavior variables, focusing on the nature of a child's cognitive, perceptual-motor, language, social and affective experiences; teacher-behavior variables, including components of teacher control, cognitive input, affective interaction with children, and evaluative feedback; and structural components of the environment, including the degree of structure of classroom materials and the extent of freedom given the child in choice of activities.

Major Findings: Data collection has been completed, and analyses are in progress. The first and already completed step in the data analysis was the establishment of inter-rater reliability among the observers. Reliability on the 120 observational categories generally ranged between 60% and 80% agreement among the three pairs of observers involved in the data collection. We are now in the process of grouping the 120 observational items into a smaller number of conceptually meaningful scales for describing salient dimensions of the preschool environment. The third step in the data analysis will be the calculation of basic frequency data on each of the experimental variables and combinations of variables. If the distribution of the data meet the necessary statistical requirements, a profile analysis based on a two-way analysis of variance will be undertaken.

Significance to Bio-medical Research and the Program of the Institute: Controversies about the relative merits of various approaches to preschool education and intervention have existed for several years. A major factor preventing resolution of these controversies is the lack of descriptive data based on direct observation of these different programs. The methodological instrument refined in this project should provide a tool for further observational research. The substantive findings from the project should provide important data about the differing experiences of children in various programs.

Proposed Course of Project: Data analyses should be completed and a report for publication prepared by the end of the current fiscal year.

Honors and Awards: None

Related Publications: Smith, M. S., and Bissell, J. S.: Report Analysis: The Impact of Head Start. Harvard Educational Review, 1970, 40, 51-104.

Serial No. HD-SB2(c)

1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: An Observational Study of Father-Child Interaction in the Early Preschool Years

Previous Serial Number: None

Principal Investigators: Frank A. Pedersen, Ph. D., Walter Brown, M.D.

Other Investigators: William Davidson, M.D., Kay Jennings, M.A., Myrna Fivel, M.A.

Cooperating Units: None

Man Years:

Total:	1.8
Professional:	1.5
Other:	.3

Project Description:

Objectives: To obtain descriptive data on the early father-child relationship in natural settings. Comparisons will be made of the behavior of fathers and mothers with their preschool age sons and daughters. Paternal behavior will be treated as an independent variable to see whether relationships exist with certain areas of child behavior. Hypotheses have been formulated regarding the father's role in the development of autonomy in early childhood. A major goal will be to develop a conceptual model for analysis of the functioning of the family as an interactive unit.

Methods Employed: The sample will consist of approximately 50 middle-class mothers and fathers and their first-born 2½ year old child. There will be two home visits in the early evening. Family interaction at a meal will be recorded by means of videotape. Following the meal, the camera will be moved to the livingroom, and each parent will be asked to engage the child in play with a toy supplied by the researchers. These structured interactions will provide data on each parent-child unit under somewhat more controlled conditions than at the meal. A third session in the laboratory playroom will focus on the child's attachment behavior to either parent and the quality of his play in a novel setting. An interview will also be conducted with the

father to obtain information about characteristic activities with the child on typical evenings and weekends.

Major Findings: The initial phase of this study has been devoted to method development. The use of videotape records for home observations is feasible. Families have reported that the camera is unintrusive, and our impression is that there is very rapid habituation to its presence. A meal at home is a completely "natural" event which has many routinized elements; at the same time, a great variety of interactions are evident involving all members of the family.

An electronic timer was devised to impose a "beep" on the audio channel at 30 second intervals. Behavior occurring within each time unit is coded by a system of categories describing to whom a message is directed, the nature and quality of the interaction, the accompanying affect, and selected responses which the message may evoke. There has been at attempt to handle some of the complexities of three-person interactions in which, for example, an intrusion into an ongoing diadic interchange may occur. These codes also deal with the child's attempts to alter the parent's behavior, as well as the more conventional notion of parental efforts to change the child's behavior.

The development of methods for the playroom observations has also been completed. In this situation a narrative record is dictated by an observer behind a one-way mirror. The amount and quality of the child's exploratory interest and play and any communication with either parent is described in a series of behavior categories. Interobserver reliability is currently being established.

Significance to Bio-medical Research and the Program of the Institute: Most of our knowledge of parental behavior in the preschool years is limited to the mother-child unit. Studies of children reared in father-absent environments, however, have suggested that there are important effects associated with paternal behavior, especially among boys. Observational data of father-child interaction are needed to understand these effects, to give psychological meaning to the father-child relationship rather than treat it in terms of a deficit concept. It is hoped that this study will lead to more sophisticated conceptual models for studying whole family interaction, i.e., father-mother-child, and its effects on the young child.

Proposed Course of Project: It is expected that data collection will continue into the winter months of FY '72. Initial coding and tabulations should be completed in this year.

Honors and Awards: None

Serial No. HD-SB3(c)

1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Reciprocal Interactions Between Mothers and Infants: The Influence of Infant Temperamental Characteristics on Maternal Behavior

Previous Serial Number: None

Principal Investigators: Leon J. Yarrow, Ph. D., Gordon Keating, M.D.

Other Investigators: William Davidson, M.D., Kay Burnes, Ph. D., Kay Jennings, M.A., Alice Abramson, M.A.

Cooperating Units: Pediatrics Service, National Naval Medical Center

Man Years:

Total:	2.50
Professional:	2.00
Other:	.50

Project Description:

Objectives: This study has two parts. The first part is concerned with developing techniques for measuring basic temperamental characteristics in young infants during the first six months, and assessing the consistency of these characteristics in infancy. The second phase of the study will be to determine the influence of these characteristics on the behavior of the mother towards the infant.

Methods Employed: The sample will consist of 35 infants and their mothers who will be studied at 4, 12, and 20 weeks of age. Subjects will be obtained through the Obstetrics Department at the National Naval Medical Center. Only mothers who have had normal pregnancies and deliveries and whose babies are evaluated as being healthy will be included in the study.

A selected number of characteristics, e.g., social responsiveness, rhythmicity, irritability, ease of being soothed, will be assessed. The methods developed consist of direct observations of the infant responses to a variety of social and inanimate stimuli in different modalities. In addition to these structured observations, an interview with the mother will aim at obtaining her perception of the infant's characteristics.

Major Findings: Pilot work is underway on the development of the observational methods and interview content.

Significance to Bio-Medical Research and the Program of the Institute: Research in early development has proceeded on the assumption that the environment acts uniformly on children with a wide variety of temperamental characteristics. Although individual differences in young infants are recognized, the role of these characteristics in mediating the effects of environmental events is not well understood. The adequacy of the conventional model of conceptualizing environmental influences acting on the organism is increasingly being questioned. This investigation will contribute to an understanding of the interaction between the child's characteristics and maternal behavior towards the child, and should lead to more adequate conceptualization of the reciprocal nature of mother-infant interaction.

Proposed Course of Project: Data collection on the first phase of the study will be completed in the next fiscal year. Planning for the second part of the study will be initiated.

Honors and Awards: None

NICHD Annual Report
July 1, 1970 through June 30, 1971
Behavioral Biology Branch

Section on Brain and Behavior

The professionals working in this Section include Dr. David Symmes, Chief of the Section, Dr. Mark Healy, Dr. Kenneth Chase, Dr. Zvi Wollberg and Dr. John Newman.

1. Dr. Symmes and Dr. Healy have continued studies initiated in fiscal year 1970 on analysing the deficits occurring in rhesus monkeys which are raised during the first few months of their life separately from their mothers and which receive a minimum of somatosensory stimulation. Continuance of profound behavioral deficits and abnormalities in these monkeys was documented using the video tape and behavioral scoring method developed last year. Three sets of electrophysiologic investigations of these monkeys have been carried out, namely, visually evoked cortical potentials, spinal reflexes and EEG recordings during sleep and waking. Visual evoked potentials recorded from the scalp were normal as were the spinal reflex measurements (which tested both the segmental reflex mechanisms and ascending pathways involving cerebellar and reticular formation motor circuits). The EEG of these animals shows abnormal patterns particularly in the slow wave stage of sleep indicating a diffuse cerebral dysfunction rather than abnormalities in the specific sensory or motor systems. No abnormalities in blood platelet serotonin levels were found.

2. Drs. David Symmes, Mark Healy and Kenneth Chase have been involved with studies on the neural correlates of multi-sensory reaction time behavior in monkeys. A system of silver-silver chloride electrodes in an epoxy resin base has been developed which is suitable for use as an implantable electrode and which permits recording of very low level, low frequency electrical signals over periods of many weeks. Computer programs for recording, cataloging and analysing such neurobiologic signals have been developed and slow wave electrical activity from animals performing a discrimination and precisely timed motor reaction have been recorded. Using these methods it has been possible to establish that different components of the slow wave electrical cortical activity are related to various aspects of this discrimination and response test. That is, some aspects of the electrical response are related to the speed or efficiency with which the response is performed, other components to the force of the response, and still other electrical components are a function of the reward the animal receives for a correct response.

3. Drs. John Newman and Zvi Wollberg are doing experiments on information processing in the central auditory system of the squirrel monkey. They have been concentrating on the use of the tape recorded vocalizations of the squirrel monkey as biologically significant stimuli in terms of which the functioning of the auditory system can be analysed. A substantial sample of single neurons in the primary auditory cortex has now been obtained.

A wide range of response characteristics has been encountered. One extreme of the range is the type of unit which responds to only one or, at the most, a small number of the squirrel monkey calls with a relatively simple response consisting of only a few spikes. The other extreme consists of cells which respond to several or perhaps all of the monkey vocalizations with a complex, highly patterned burst of action potentials. The role that various components of the vocalizations play in eliciting unitary firing has been studied by electronically removing various components of the vocalizations as the vocalizations are presented. This has revealed that the response of a given unit may be due to two or three key features of a given call and that other features of the call make relatively little difference to the cell's responsiveness. Thus, in the primary auditory cortex, single neurons seem capable of signalling the occurrence of certain key features of vocalizations in these animals.

Section on Neurobiology

The professionals in this section include Drs. Phillip Nelson, Gerald Fischbach, John Peacock, Harold Gainer, Donald Shapiro, Bruce Schrier and Herbert Levitan.

1. Dr. Harold Gainer has been working on the neurobiology of the land snail. He has succeeded in adapting the micro disc electrophoresis method for analysis of cell proteins so that a single snail neuron can now be dissected out and analysed for the molecular weight distribution of its proteins. This method has been used for characterizing the similarities and differences between the protein synthetic patterns in a group of six identified cells. Particularly, one of these cells, cell #11, has been studied intensively because of very large changes in its activity during the course of hibernation and activation. On the basis of the large granules revealed by electron microscopy, this cell can be classified as a neurosecretory cell. On disc electrophoresis a prominent peak at a molecular weight of about 5000 daltons is observed when cell #11 from an active animal is analysed but this peak is completely absent in the hibernating animal. This peak is unique to this cell among those studied. Thus, this cell has a characteristic electrophysiologic pattern which has now been described in detail, has a characteristic electron microscopic appearance and a cell specific protein which is qualitatively changed as a function of the state of the animal.

2. In vitro tissue culture systems. Drs. Nelson and Peacock have continued collaboration with the Laboratory of Biochemical Genetics of the National Heart and Lung Institute on the study of neuroblastoma cell cultures. Two clones of neuroblastoma cells have been looked at in detail with regard to electrical excitability and have been found to differ very markedly from one another. One line of cells has a relatively weak electrical activity even under conditions of maximum differentiation of the line. Another line of cells has a larger percentage of cells which stop dividing and differentiate, both morphologically and electrophysiologically. A number of culture conditions have been found to affect both morphology and electrical activity in this latter line of cells. When this line is allowed

to grow to confluency, cell division is markedly reduced, electrical activity is increased and development of cell processes is increased. A selection technique was devised by Dr. Minna involving the use of the mitotic inhibitor, aminopterin, which similarly promoted cell differentiation. Thus, in the neuroblastoma cultures both clonal differences and differences between different states of a given culture provide a wide spectrum with regard to the expression of the property of electrical excitability. Neuroblastoma cells have been found to respond to application of acetylcholine with inhibitory or excitatory changes in membrane potential. Atropine and curare can partially or completely block these responses. The neuroblastoma thus provides a good model system for the study of cell process formation, electrical excitability and chemosensitivity. A hybrid line of cells formed between neuroblastoma cells which generate action potentials and a line of fibroblast cells which do not has been studied with electrophysiologic methods. This hybrid line was formed by Dr. John Minna in the Laboratory of Biochemical Genetics. These hybrid lines have provided an additional tool for the analysis of membrane mechanism such as electrical excitability and chemosensitivity. The action potential mechanism provides a marker for the neuroblastoma cell line and study of the fibroblast line has led to the discovery of a new electrical membrane mechanism which serves as a marker for the fibroblast cell line. The fibroblasts have relatively low membrane potential, a very low resting membrane permeability to potassium and respond to large stimulating pulses of current with a prolonged, marked increase in membrane permeability to potassium. In the hybrids between these two lines, the various clones obtained following the hybridization present a spectrum of combinations of the two types of membrane properties. In particular, one clone was obtained which exhibited well-developed action potentials and had well-developed cell processes. This line was thus rather similar to the parent neuroblastoma cell line. A second clone from the hybridization procedure had markedly less prominent action potentials, relatively few processes and was much more like the fibroblast cell line. Nonetheless, this line showed some evidence of the neuroblastoma-like active membrane response. Thus, the hybrids represent another method for obtaining a wide range of intensity of expression of neuronal characteristics.

3. Dr. Fischbach has been examining normal spinal cord and muscle tissue cultures for electrophysiologic and biochemical activity. Dr. Fischbach has examined the electrophysiology of nerve cells from chick spinal cord as they develop from dissociated cultures and establish functional, synaptically linked nets of neurons. He has also looked at the interaction between nerve cells developing from such dissociated cultures as they establish functional neuromuscular contacts with muscle cells from embryonic chick. Excitatory and inhibitory synaptic potentials occur in the neurons and cholinergic synapses are formed between the nerve and muscle cells with most of the characteristics of normal neuromuscular synapses. The effect of innervation of muscle by cultured neurons appears to effect the pattern of distribution of acetylcholine sensitivity along the muscle fibers thus providing a model for trophic interaction between nerve and muscle. A number of techniques including the use of metabolic inhibitors have been developed to optimize the growth of the neurons, to minimize the

contamination of connective tissue and glia elements, and to maximize the degree of synaptic interaction between nerve cells and other nerve cells and between nerve cells and muscle.

4. Dr. Schrier and Dr. Shapiro have investigated the biochemical functioning of long term tissue cultures formed from fetal mammalian brain cells. Cholinesterase and choline acetylase determinations have been used as an index of maturation and differentiation of these cultures. Such factors as cell density, culture growth rate and the influence of different culture media on the development of enzyme activity have been studied in an attempt to optimize culture conditions.

In Summary:

The Section on Brain and Behavior has nearly completed a pilot study of some behavioral, electrophysiological and biochemical consequences of early sensory and social deprivation. Studies on electro-cortical concomitants of higher cognitive function are in progress. Substantial information has been obtained regarding the analysis of complex, biologically significant acoustic signals at the level of the primary auditory cortex in a primate.

In the Section on Neurobiology a small number of cells in a primitive (snail) nervous system have been intensively analysed by electrophysiologic, biochemical and electron microscopic techniques. A novel technique for separation and identification of proteins, applicable to single neurons has been developed. Electrophysiologic investigation of a number of clones of neuroblastoma cells under various culture conditions has been carried out. The technique of somatic cell hybridization has been utilized (in collaboration with the Laboratory of Biochemical Genetics, NHLI) in dissecting some important electrophysiologic parameters. Normal fetal brain, spinal cord and muscle have been maintained for prolonged periods in vitro following dissociation into single cells. Intact synapses have been demonstrated electrophysiologically and increasing levels of neuronal marker enzymes have been shown to occur with time in vitro.

The efforts of the Neurobiology Section are largely directed, at this point, toward developing the appropriate in vitro or small intact systems for molecular and ultrastructural analysis. It is already clear that a wide variety of neurobiologically interesting phenomena will be available for analysis in these systems.

It is reasonable to assume that these neurobiologic phenomena contribute importantly to the operation of the intact nervous system and that an understanding of the molecular and structural basis for these phenomena will greatly increase our understanding of brain function.

Serial No. HD-BB2
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of CNS functioning in isolation reared monkeys.

Previous Serial Number: HD-BB2

Principal Investigator: David Symmes, Ph.D.

Other Investigators: Mark Healy, M.D.

Cooperating Units: Childrens Hospital, Department of Neurology
Washington, D.C.

Man Years:

Total:	1.5
Professional:	1.0
Others:	.5

Project Description:

Objectives: To describe alterations in CNS function, if any, which are associated with the isolation-reared syndrome in monkeys.

Methods Employed: The group of four experimental and four control monkeys with which this project was begun are still under study. The animals are about two years of age at the end of fiscal year 1971, and investigations have been carried out since they reached age 9 months in the following areas: EEG; evoked potentials; H-reflexes; platelet serotonin; and behavior. The last has been especially extensive and a video taped sample of behavior of all animals singly and in groups has been analysed and stored as development progressed. Problem solving behavior and the effects on motor coordination of displaced visual fields have also been studied.

Major Findings: No differences between the groups have been found in evoked potentials, reflex status, or serotonin levels. Abnormal slow wave sleep EEG records are characteristic of the isolation reared animals. Behaviorally, a pattern of strikingly abnormal autostimulatory and sterotyped behaviors has been quantified, and the fixed components of this syndrome have been identified.

Significance to Biomedical Research and the Program of the Institute: The severity and persistence of the behavioral abnormalities associated with isolation rearing are well documented, yet little or no data exists on the

neurochemical or neurophysiological mechanisms which may be causally related. Some insight into these mechanisms could have wide applicability in the diagnosis and treatment of certain childhood disorders, particularly autism.

Proposed Course: To complete present behavioral and EEG studies; then to implant the animals with multi-lead depth electrodes (the cranium now being sufficiently calcified to give a reasonable chance of secure mounting) and to examine correlations between depth recordings and behavior. The present schedule calls for sacrifice of this pilot group in fiscal year 1971 with verification of electrode location and histochemical studies as indicated.

Honors and Awards: None

Publications: None

Serial No. HD-BB1
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neural correlates of multi-sensory reaction time behavior in monkeys.

Previous Serial Number: HD-BB1

Principal Investigator: David Symmes, Ph.D.

Other Investigators: Mark Healy, M.D.
Kenneth Chase, M.D.

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	2.0
Others:	.5

Project Description:

Objectives: To develop an understanding of the origin and significance of slow cerebral potentials associated with cognitive processing through the use of infrahuman primate models.

Methods Employed: During fiscal year 1971 progress in this project has proceeded in 3 areas: 1) development of suitable recording techniques. The critical nature of the electrode/tissue interface for slow potential recording has necessitated a great deal of time and effort in the development of this technical aspect. A system of silver-silver chloride epoxy based implantable electrodes has been worked out which permits high quality recording of waveforms with time constants of 9 seconds. This system is in use. 2) Design and fabrication of a sophisticated behavioral testing apparatus including a remotely controllable lever which gives computer compatible force and position voltage outputs. 3) Utilization of a PDP-12 computer to acquire, store, and segregate individual lever pulling responses (reaction times). Programs have been developed which greatly increase experimenter efficiency in handling the many variables which are or may be causally related to slow potentials recorded from the cortex; i.e.: reaction time, hand in use, force exerted, duration of unpredictable foreperiod, and the spatial distribution of relevant potentials.

Major Findings: Individual trials consisting of an appropriate timed lever pulling and releasing response accompanied by four channels of artifact-free EEG recordings are the basic datum of this project. Approximately 1500 such trials from four trained monkeys have been stored via the computer and are being analysed. Data is presently being gathered from a fifth monkey, and one animal proved to be unsuitable after extensive training and electrode implantation due to its excessive movement in the restraining chair. The results which have emerged from analysis so far suggest 1) that slow potentials are prominently and consistently distributed over the cortex during such behavioral performance; 2) that at least superficially these potentials resemble those recorded from human subjects in similar situations; 3) that there exist multiple origins of complex slow waves, including components relating to reaction time, to muscular tension or force, and to the acquisition of a desired reward.

Significance to Biomedical Research and the Program of the Institute: The behavior and cerebral recordings studied in this project are analogs of procedures widely used in adults and children to assess CNS function. The CNV wave (contingent negative variation, after Walter) has been described as altered in several types of psychiatric and mentally retarded patients, and these studies will increase our understanding of its origin and physiological significance.

Proposed Course: To continue as above: to record unit firing in awake, chronically prepared monkeys during behavioral performance.

Honors and Awards: None

Publications: None

Serial No. HD-BB4
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Information processing in the central auditory system of mammals.

Previous Serial Number: HD-BB4

Principal Investigators: John D. Newman, Ph.D.
Zvi Wollberg, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	2.0
Others:	0.0

Project Description:

Objectives: This project is concerned with the processing of complex sounds by neurons in the primate cerebral cortex and related brain structures. Special attention is being paid to sound used by primates in intra-specific communication to determine which acoustic features of these sounds are used by the brain to distinguish between the various elements of the communication repertoire.

Methods Employed: Action potentials are recorded from single cells in the primary auditory cortex of unanesthesized squirrel monkeys. Detailed analysis of the responses evoked in these units by tape recorded squirrel monkey vocalizations is made possible by a system that has been devised for repetitive presentations of the squirrel monkey vocalizations. This is used in conjunction of the special display with averaging of the cell responses to these vocalizations. Selected segments of the vocalizations may be removed by an electronic switch so that the importance of different components of the calls can be evaluated.

Major Findings: 1) More than 80 percent of the 213 units tested responded to one or more vocalizations. 2) A wide variety of response patterns was observed. The response patterns, however, can be categorized according to the selectivity of the responses and the temporal complexity of the discharge patterns. At one extreme are cells which respond to one or only a few call types with a simple response pattern. At the other extreme are

cells which respond to many or all of the vocalizations in our catalog with temporally complex response patterns. Intermediates between these extreme types were also frequently encountered. 3) The use of the electronic switch has permitted us to determine in some cases which specific portions of a given vocalization are necessary to produce a given neuronal discharge pattern. We have been able to eliminate one or more parts of a neuronal response by selective removal of part of a call. In some cases we have found that the nature of the neuron's response can be the result of the interaction between different portions of a given vocalization. 4) Preliminary results indicate that some neurons in the auditory cortex are selectively responsive to some but not all varieties of the same call type.

Significance to Biomedical Research and the Program of the Institute: This project has identified neurons in the auditory centers of the primate cerebral cortex which are responsive to sounds used as species-specific communication signals. Analysis of the discharge pattern indicates that many of these neurons are sensitive to specific features of these sounds and that different neurons respond to different acoustic cues within these sounds, such as temporal pattern, spectral components and frequency shifts. These same cues are also important in human speech recognition and it is hoped that these results have some relevance to the problem of mechanisms in the human brain related to the processing of speech sounds.

Proposed Course: Two major goals of this project are 1) determination of the acoustic features within communication related sounds which are analysed by neurons in the auditory region of the primate cerebral cortex and 2) the acoustic features within these sounds which are used by the behaving animal to distinguish between the various animals of its vocal repertoire. To this end we are developing methods to generate complex sounds which imitate one or more features of a natural vocalization and to use controlled manipulations of these synthetic vocalizations both in studies of single neurons and in studies of behaving monkeys trained to discriminate between various complex sounds.

Honors and Awards: None

Publications:

Winter P., and Funkenstein, H.H. The auditory cortex of the squirrel monkey: Neuronal discharge patterns to auditory stimuli. Proc. 3rd int. Congr. Primat. 5: 1971 (In press).

Serial No. HD-BB8
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Correlative neurochemical and electrophysiological analyses of individual, identified neurons in the brain of the land snail, Otalea lactea: The effects of hibernation and estivation.

Previous Serial Number: HD-BB8

Principal Investigator: Harold Gainer, Ph.D.

Other Investigators: Thomas S. Reese, M.D.

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	1.0
Others:	0.1

Project Description:

Objectives: To study the neurochemical and electrophysiological changes which occur in specific identified central neurons of the land snail as a consequence of hibernation of the whole animal, and endogenous diurnal rhythms.

Methods Employed: A major effort has been directed towards developing a microdisc polyacrylamide electrophoresis technique in sodium dodecyl sulfate for the analysis of protein synthesis in single neurons. This method which has been completed (a paper on the micromethod is now in preparation) consists of a disc system cast in 580 micron diameter capilary tubes. The micromethod is comparable to conventional size SDS gels in its molecular sieving characteristics, and is sensitive to as low as 5×10^{-10} grams of protein. The sensitivity of the method is even greater when it is used in conjunction with isotopically labeled proteins, and has been found satisfactory for the analysis of protein in single cells.

A topographic map of the largest (> 100 microns) neurons of the land snail brain (Otala lactea) has been prepared, and the electrophysiological characterization of these individual, identified neurons by conventional microelectrophysiological methods is in progress. Methods for aestivating

and hibernating the animals have been worked out, and correlative electrophysiological and protein synthesis studies using isotopically labeled amino acids have been conducted on neurons from dormant and active animals.

Electron microscopy of single, identified neurons has been done with the collaboration of Dr. Thomas Reese.

Major Findings: Five identified neurons (Cells #7, 9, 10, 11, and 12) have been studied intensively. These show different electrical properties (e.g., cells 7, 9, and 12 are inactive without stimulation, cell 10 is a very active beating pacemaker-2/sec., and cell 11 is a very active bursting pacemaker) and different responses to aestivation (i.e. no change in cells 7, 9, and 10, depolarization and decrease in membrane resistance in cell 12, and loss of bursting pacemaker activity, hyperpolarization, and decreased membrane resistance in cell 11). Cell 11 has been conclusively shown to have endogenous pacemaker activity by observing a bursting rhythm in a completely isolated cell. The pacemaker properties of this neuron have been studied in detail, and the pacemaker potential appears to be due to an oscillatory change in sodium permeability while the action potential appears to involve both an increased sodium and calcium conductance. Electron micrographs of cell 11 show it to be a neurosecretory cell with a very heavy concentration of neurosecretory granules (2000-2500A) and free ribosomes. Cell 11 also shows seasonal and diurnal variations in its electrical behavior consistent with its neurosecretory role.

Studies of the protein synthesis of these 5 identified neurons have revealed neuron-specific patterns of synthesis. In general, the silent cells (7, 9, and 12) had a similar profile of incorporation of ^{3}H-l-leucine after 20 hours incubation, the beating pacemaker (cell 10) had a larger contribution of synthesis from proteins with molecular weights of less than 25,000 daltons, and cell 11 showed an enormous synthesis of low molecular weight proteins, in particular, it had a peak of synthesis at 5000 daltons representing some 20-25% of the total protein synthesis of the cell. This 5000 dalton peak represented a neuron specific protein found only in an active cell 11. In the dormant animal this peak of activity was specifically absent in cell 11. Since the 5000 dalton peak appears in the neurosecretory neuron R_{15} in another gastropod species (Aplysia californica) but not in R_2 (non-neurosecretory cell), it is suggestive that this protein (or class of proteins) plays some role in neurosecretion, either as the neurohormone or as a carrier protein for the neurohormone.

Significance to Biomedical Research and the Program of the Institute: Current approaches to neural function emphasize the heterogeneous nature of neuronal populations with regard to their behavioral, electrical, and biochemical properties. The approach described above can begin to sort out the relevant biochemical changes taking place in the specific neurons within a natural population of neurons which are uniquely involved in functional changes. The dormant versus active neuron could provide a model system for the study of the control of specific neuronal protein synthesis. Finally, this methodology and approach can be effectively applied to the analysis of protein synthesis changes in neurons of a developing nervous system, and hence, this project is highly relevant to the long-term aims of the Institute.

Proposed Course: In order to study control of protein synthesis (particularly of the 5000 dalton peak) in cell 11, it is necessary to develop an *in vitro* method by which a dormant cell 11 can be converted into an active one. The blood of snails recently activated from dormancy will be collected, and activation factors from it will be looked for. Once such a factor is obtained, a search for the synthesis of the appropriate m-RNA can proceed, and detailed studies of control mechanisms are possible. Another course is to see what effects, if any, long-term selective stimulation of the cell (synaptic or direct) has on its pattern of protein synthesis. Presumably long-term biochemical changes in neurons will be induced by their specific electrical activities, and the coupling of these two different forms of information could be studied in this system. Finally, the micromethod will be applied to various vertebrate neurons in tissue culture, with the aim of finding neuron-specific patterns. Such a finding would greatly facilitate the attempt to produce a homogeneous population of differentiated neurons in culture by providing a simple and specific assay for homogeneity.

Honors and Awards: None

Publications: None

Serial No. HD-BB5
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electrophysiology studies of neurons in vitro.

Previous Serial Number: HD-BB5

Principal Investigators: Phillip G. Nelson, M.D., Ph.D.
John H. Peacock, M.D.

Other Investigators: None

Cooperating Units: Laboratory of Biochemical Genetics, National Heart and Lung Institute

Man Years:

Total:	2.5
Professional:	2.0
Others:	0.5

Project Description:

Objectives: To develop neural tissue culture systems so that the molecular basis and the control mechanisms for regulating a variety of neurobiologic phenomenon will be investigated.

Methods Employed: Neuronal tumor cells have been cloned and somatic cell hybrids between neuronal and non-neuronal cell lines have been developed. Electrophysiologic techniques of intracellular recording and micro-iontophoresis have been used to test the passive membrane properties, the electrical excitability and chemosensitivity of these lines of cells. Computer programs have been developed to allow on-line analysis of cell membrane electrical properties.

Major Findings: 1) Different clones of neuroblastoma cells exhibit different degrees of electrical excitability. 2) Electrical excitability is a function of culture conditions such as state of mitotic activity and cell density. Selection of non-dividing cells by addition of aminopterin to the medium results in a culture, well differentiated morphologically and electrically. 3) Hybrid cell lines containing the genomes of neuronal and fibroblast (L-cells) lines exhibit a spectrum of morphologic and electrical properties. Some hybrid clones resembled the neuronal line more closely and some clones resembled the fibroblast line. Process (neurite)

formation and action potential generation can be even more marked in the hybrid than in the parent neuroblastoma. Other hybrids exhibit little or no process formation and very weak electrical excitability. 4) Neuroblastoma cells respond to iontophoretically applied acetylcholine with either excitatory depolarizing changes in membrane potential or with inhibitory hyperpolarizing potentials. Atropine can partially or completely block hyperpolarizing membrane potential changes and curare also has some blocking action on the acetylcholine evoked change in membrane potential. Wide variation in chemosensitivity is seen in different cells in a given culture; thus far the factors regulating the chemosensitive property remain obscure. 5) A new active electrical response in the fibroblast line has been discovered which consists of a large increase in membrane permeability to potassium.

Significance to Biomedical Research and the Program of the Institute: The neuroblastoma and hybrid cell lines represent a favorable preparation for exploring the properties of electrical excitability and chemosensitivity. Knowledge as to the molecular basis for these properties and the processes which regulate their expression would add greatly to the understanding of nervous system function.

Proposed Course: The wider range of neuroblastoma clones of hybrid lines and of culture conditions will be explored in an attempt to determine the factors controlling chemosensitivity. The neuroblastoma and hybrid lines will be interacted with other tissues such as muscle in an attempt to produce functioning synaptic connections between the continuous lines of cells and other tissues. Normal mouse spinal cord will be cultured and an attempt to produce hybrids between this material and continuous cell lines such as the L-cell will be made. The goal of the above procedures is to obtain a series of cell lines with the capacity to form synaptic connections. Preliminary results on the protein synthetic activity of these lines of cells will be expanded.

Honors and Awards: None

Publications:

Minna, J., Nelson, P., Peacock, J., Glazer, D., and Nirenberg, M.: Genes for neuronal properties expressed in neuroblastoma x L cell hybrids. Proc. Nat. Acad. Sci. 68: 234-239, 1971.

Nelson, P. G., Peacock, J. H., Amano, T., and Minna, J. Electrogenesis in mouse neuroblastoma cells in vitro. J. Cell. Physiol. 77: 1971 (In press).

Nelson, P. G., Peacock, J. H., and Amano, T. Responses of neuroblastoma cells to iontophoretically applied acetylcholine. J. Cell. Physiol. 77: 1971 (In press).

Serial No. HD-BB6
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of excitability and synapse formation in nerve and muscle cells in tissue culture.

Previous Serial Number: HD-BB6

Principal Investigator: Gerald D. Fischbach, M.D.

Other Investigators: Phillip G. Nelson, M.D.
Harold Gainer, Ph.D.
John Heuser, M.D.
Mark Dichter, M.D.
Stephen Cohen

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.0
Others:	0.5

Project Description:

Objectives: Our long range goal is to study the underlying biochemical and genetic mechanisms of synapse formation in a simple system. Although a great deal is known about the selective permeability, non-linear responses and receptor function of membranes of excitable cells in adult animals, nothing is known concerning how, in the course of cell differentiation, these unique properties arise. This information is essential for understanding the development of communication between excitable cells. A second objective, now that synapse formation has been demonstrated (see below) in our in vitro system, will be to determine the precise sequence and range of electrophysiological events during formation of neuron-muscle and neuron-neuron synapses, the range of synaptic types evident in culture and the degree of specificity exhibited by such functional contacts. Answers to these questions will suggest what underlying biochemical and biophysical events are causal mechanisms. In addition, knowledge of the first stages of synaptic function will, perhaps, elucidate the mechanism of quantal release of transmitter characteristic of adult chemical synapses. Third, the biochemistry and electron microscopy of membrane and cytoplasmic constituents of cloned muscle and "purified" nerve cultures will be investigated. Finally, the effect of long-term, imposed patterns of impulse activity on the physiologic, biochemical and structural properties of the cells will be studied.

Methods Employed: Conventional cell and organ culture techniques have been employed. Nutritional and other factors to optimize cloning techniques will be explored further. Microelectrode techniques include intracellular recording and stimulation and electrophoretic injection of chemicals into and on the surface of single cells. The examination of single cells is made possible by direct visualization of the cells and microelectrodes with an inverted phase contrast microscope. Analog - Digital computer techniques, autoradiography and protein biochemistry assays are being developed.

Major Findings: Cultures of muscle and spinal cord cells relatively free of background connective tissue cells have been obtained by the addition of mitotic inhibitors to the culture medium. Electrophysiological experiments are greatly facilitated in these "clean" preparations.

Neuromuscular junctions formed in vitro (between thoracic spinal cord cells and pectoral myotubes) are similar to adult junctions: transmitter release is quantal, the post-synaptic potential is reduced by curare and Mg ions and is reversed in polarity when the muscle membrane is depolarized to -10 to 0 mV. Large (5-20 mV) and small (5-2 mV) synaptic potentials occur spontaneously. The former are blocked by tetrodotoxin and, therefore, are probably caused by spontaneous firing of an innervating neuron; the latter are not affected by the toxin and therefore probably represent true spontaneous leak of transmitter quanta (miniature endplate potentials or mepp). The mean frequency of mepps and the mean quantum content of stimulus evoked endplate potentials (epps) are low compared to adult neuromuscular junctions. In many cases the mepp frequency increased dramatically following a single stimulus of the neuron. In a few cases, direct electrical interaction between nerve and muscle cells has been observed. Muscle fibers may be innervated by a given neuron at more than one point and some data are consistent with innervation of one muscle cell by more than one neuron.

Spinal cord cells probably exert trophic influences on muscle fibers in vitro. Relative peaks of sensitivity to iontophoretically applied acetylcholine (ACh) and characteristic accumulations of the enzyme acetylcholinesterase have been found along innervated fibers. Control experiments are not yet complete.

Interneuronal excitatory and inhibitory synapses have been detected in the spinal cord cultures. Dorsal root ganglion cells grown in an identical manner retain their distinctive morphology and exhibit characteristic, prolonged action potentials.

Significance to Biomedical Research and the Program of the Institute: The mechanism of synaptic formation is a basic question in embryology. Formation and interuption of synaptic coupling between nerve and various nerve and muscle cells has profound structural and metabolic consequences. The tissue culture technique offers considerable hope for analysing this striking histologic phenomenon. Modification of synaptic function by changes in activity pattern would help in understanding mechanisms of neuronal plasticity or learning. Before asking the relevant biochemical questions, the basic electrophysiological phenomena must be understood. Tissue culture has provided a convenient system in this regard.

Proposed Course: Additional modifications of culture conditions will be explored in an attempt to further isolate different populations of nerve and muscle cells. Density gradient centrifugation will be used in this regard. "Purification" will be assayed with electrophysiologic, and biochemical (choline acetylase and acetylcholinesterase) techniques.

In the nerve-muscle cultures, mepp frequency and amplitude histograms, mean quantum content during repetitive stimulation and the occurance of multiple innervation and direct electrical coupling will be studied as a function of time to determine if a clear "developmental program" is followed. These phenomena will be correlated with synaptic ultrastructure (with Dr. J. Heuser). A PDP-12 computer will be used to follow the statistics of transmitter release.

The specificity of synapse formation will be determined by culturing various muscles with different spinal cord segments and different mixtures of spinal cord with dorsal root ganglion cells. If specificity is demonstrated, the time of specification will be determined by initiating cultures from different age embryos and by studying cell division in vitro. In addition restriction of different types of synapses to certain areas of the postsynaptic neuron (dendrites vs. soma) will be tested.

The occurrance and nature of trophic interaction between nerve and muscle will be continued. Mechanisms will be sought in axoplasmic and transsynaptic flow of molecules and in enzyme induction - both pre and postsynaptic. Protein synthesis in single cells will be studied with Dr. H. Gainer. Trophic influences of dorsal root ganglion cells on muscle and the requirement of synaptic contact will be tested.

The ionic basis of passive and active membrane phenomena of the various neurons will be studied with Dr. M. Dichter. The type and detailed topography of chemosensitivity of these cells and the principles governing simple circuit formation between them will also be undertaken.

Attempts to hybridize central nervous system cells with another dividing line of cells will be made with Drs. Nelson, Peacock and Levitan. Success might lead to an analysis of genetic mechanisms underlying the phenomenology discussed above.

Honors and Awards: None

Publications:

Fischbach, G. D.: Synaptic potentials recorded in cell cultures of nerve and muscle. Science, 1970, 169: 1331-1333.

Fischbach, G. D., Nameroff, M. and Nelson, P. G. Electrical properties of chick skeletal muscle fibers developing in tissue culture. J. Cell Physiol. 1970 (In press).

Serial No. HD-BB3(1)
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Morphologic and biochemical studies of fetal brain cell cultures.

Previous Serial Number: New project

Principal Investigator: Bruce K. Schrier, M.D., Ph.D.

Other Investigators: Donald L. Shapiro, M.D.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To study, by biochemical and electrophysiologic methods, selected populations of identified neurons and neuron-glia combinations in a controlled environment. To this end, a culture system utilizing fetal rat brain cells is being characterized. We hope to determine 1) whether normal immature neurons are capable of multiplication in culture, 2) factors necessary to neuronal differentiation in culture, and 3) ways to obtain purified populations of neurons and glial cells.

Methods Employed: Most of the cultural and biochemical methods were developed while the principal investigator was a member of the Laboratory of Biochemical Genetics of the National Heart and Lung Institute (manuscripts in preparation: S. Wilson, B. Schrier, J. Farber, A. Blume and M. Nirenberg, "Sensitive Radiochemical Methods for Measuring Enzyme Activities in Mammalian Cell Culture Extracts"; B. Schrier, J. Farber, R. Rosenberg, E. Thompson, S. Wilson and M. Nirenberg, "Biochemical and Morphologic Changes Associated with Growth of Normal Mammalian Brain Cells in Culture", B. Schrier and M. Nirenberg, "Effects of Prolonged Culture, Subculture, and 1,1,3-tricyano-2-amino-1-propene on Fetal Rat Brain Cells"). In addition, the effects of different culture media and sera are being investigated. Certain histochemical tests for neuronal markers will be applied. The system will be evaluated for neuronal differentiation capability using variations in fetal age, plating density, subculture time, type of culture medium, etc.

Gradients of dense material (Ficoll, BSA, etc.) will be used to purify individual cell types from cultured or freshly dissociated material. An evaluation of the production by these preparations of 3'-5'cyclic AMP in response to hormones is planned.

Major Findings: Cells obtained from fetal rat brains by dissociation may be grown in culture for at least 2 months. During this time there is an apparent differentiation of neurons, as measured by the cultural levels of choline acetyltransferase (CAT), the enzyme which forms acetylcholine. The rate at which maximum levels of the activity are reached and the total enzyme production are affected by variations in plating density, age of fetus, and type of culture medium. Preliminary results indicate that the level of neuronal differentiation attained is limited by some culture factor, such as crowding and that this limit may be released by subculturing or otherwise limiting crowding.

Significance to Biomedical Research and the Program of the Institute: The molecular basis of neuronal properties such as excitability and chemosensitivity remains unknown. The development of tissue culture lines of neurons differing in these properties may allow the demonstration of the biochemical correlates of these different neuronal properties. The probability seems high that knowledge of the factors governing neuronal development and function would have high relevance to the understanding of the normal development of mental capabilities as well as factors involved in mental retardation.

Proposed Course: We will continue to evaluate fetal brain cultures until we are able to predict the system and know the conditions for optimal differentiation. Meanwhile, we are attempting to develop methods for purification of individual brain cell populations. We also will use histochemical and electrophysiologic techniques to determine which cells in the preparations are neuronal, and as correlative data to the enzymatic criterion for neuron purification. An evaluation of cultural cyclic AMP levels in response to hormones is planned.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1970 through June 30, 1971
Laboratory of Biomedical Sciences

The Laboratory of Biomedical Sciences conducts basic research in molecular genetics, developmental enzymology, intermediary metabolism, physiological controls, developmental pharmacology, and molecular structure. The objective of these investigations is the elucidation of the molecular basis for selected types of mental and physical developmental pathology.

The Section on Molecular Genetics is investigating three major processes in the transfer of genetic information. The first of these involves the mechanism by which a set of viral genes may be inserted into the chromosome of a host cell and continuously replicated with the host genome; the second involves the mechanism by which genetic information is translated into functional cellular proteins and the mechanism by which this process is regulated; and the third involves the mechanism by which a population of cells is modified to accomplish specific functions, for example, the synthesis of specific antibodies. The work is relevant to our understanding of the fundamental basis of genetic disorders and of defects which may arise as a result of post-conceptual gene damage. It is also relevant to the problem of oncogenesis in that several lysogenic viruses have been implicated in the induction of neoplastic disease in animals.

Recent work in the Section has delineated genetically the mechanism involved in the insertion and excision of the phage λ into and from its host's genome. By removing the usual attachment site for this phage, it has also been possible to insert into many points in the host genome and thus to produce a wide variety of phage containing small portions of the host chromosome. These are vital to subsequent studies requiring a relatively purified set of genes and should prove extremely useful to the field in general.

Further, two cell-free protein synthesizing systems have been derived from bacteria and from Krebs II ascites cells. Both of these appear to translate phage and viral mRNA under conditions which give rise to authentic viral proteins. The bacterial system has been used to show that repression of a phage function by its own coat protein prevents translation of the first two codons of the repressed function. Thus the site of action of this repressor has been determined. Use of antibody inhibitors of protein synthesis and specific degeneracy classes of tRNA permitted the elucidation of a very narrow range of nucleotide sequences corresponding to the initial region of the repressed cistron. This sequence very likely contains an operator-like region serving as a receptor for the regulatory protein.

Studies involving the Krebs ascites cell-free system show a striking protein synthetic response to very small amounts of encephalmyocarditis virus (EMC) mRNA. A clear species specificity with respect to tRNA's derived from mammalian sources has been observed. Preliminary experiments indicate that the system will translate mRNA derived from myeloma cells. Hopefully, the system may be used to quantitate and possibly to isolate immunoglobulin mRNA. Related studies using a cell culture system have shown that single cells challenged with an antigen containing two hapten determinates will produce

antibody to only one of these. Over the next year this antibody producing cell-culture system should provide considerable insight into the mechanism by which cells are committed to a specific antibody response.

In the Section on Developmental Enzymology interest centers on the separation, purification, and characterization of enzymes from the human placenta and from fetal tissues in both normal and abnormal pregnancies. A study of the properties of pregnancy-associated enzymes from normal and abnormal gestations may give information about the etiology of reproductive disorders such as spontaneous or habitual abortions, stillbirths, or molar pregnancies.

Six placental enzymes are under study. Glucose dehydrogenase has been purified 5000-fold and has been characterized with regard to pH optimum, substrate affinities, and molecular weight. Beta glucuronidase and hexosaminidase have been partially purified; the isoenzymes of hexosaminidase have been separated by gel adsorption. Methods have been devised for demonstrating isoenzyme patterns of these three enzymes from human placenta and gestational serum. During the growth and development of an organ, the enzymes of that organ may show developmental variations in isoenzyme pattern, in total activity, or in both. Since little is known about the ontogeny of placental enzymes, we are studying the qualitative and quantitative changes in six enzymes in placentas obtained from eight weeks to term. Significant changes in levels of activities occur in half of the enzymes studied. In addition, striking changes in isoenzyme patterns and in total enzyme activities were observed in two placentas associated with "blighted" ovum abortions. This is the first study demonstrating qualitative and quantitative changes in enzyme activity in abnormal products of conception.

Investigators in the Section on Intermediary Metabolism develop laboratory analogs of mental retardation through the use of specific chemical agents and enzyme inhibitors, investigate the biosynthesis and degradation of macromolecules in neural tissue, elucidate mechanisms of transport of small molecules into and out of the brain, and evaluate the effects of excesses or deficiencies of intermediary metabolites on cellular differentiation and organogenesis (particularly of the brain). The biochemical correlates of abnormal nervous activity in the animal model systems are studied and are used as the basis for developing screening procedures for detecting enzyme defects in mental retardation.

Among recent advances made by the Section are: (1) demonstration that p-chlorophenylalanine and phenylalanine treatment in the newborn rat produces a persistent neuropathology and an irreversible deficit in the adult rat's behavior in a number of test situations; (2) purification of bacterial phenylalanine hydroxylase to near homogeneity and a study of its stimulation by bovine serum albumin; (3) characterization of the first and second enzymes of pteridine biosynthesis in Comamonas sp. and characterization of the first intermediate as a new compound, a cyclic phosphate of neopterin; (4) demonstration that rat brain uses uridine in preference to orotic acid as a precursor of pyrimidines--a finding which indicates that de novo pyrimidine synthesis plays a relatively minor role in brain nucleic acid metabolism; (5) demonstration that young rats have higher levels of biosynthetic enzymes and much lower levels of degradative enzymes in brain tissue than do adults;

(6) complete purification and characterization of a phenylalanine binding protein from Comamonas and implication of the amino group of lysine as a component of the binding site; and (7) purification of an intracellular inhibitor for an intracellular proteinase.

The Section on Physiological Controls conducts research on the regulation of cellular and organ metabolism by hormones, nutrients, and neural mechanisms. Current areas of interest are: (1) the role of cyclic AMP (adenosine 3',5'-monophosphate) in regulating the intracellular effects of polypeptide hormones and biogenic amines and (2) the mechanism by which nutrients and neural stimuli modify the metabolism or action of cyclic AMP. Processes mediated by cyclic AMP are known to be important in regulating cellular growth, differentiation, and cellular metabolism at many phylogenetic levels.

During the past year, work in the Section has (1) continued to characterize mechanisms by which glucose and cyclic AMP interact to control liver glycogenolysis and protein synthesis, (2) further defined the role of α-adrenergic receptors in the control of liver phosphorylase activity and cyclic AMP generation, (3) accumulated further evidence to support the hypothesis that serotonin N-acetyltransferase is principally involved in the regulation of pineal serotonin levels and melatonin synthesis, and (4) described a regulation of pineal phospholipid metabolism by norepinephrine and α- and β-adrenergic receptors.

An extremely sensitive assay for cyclic AMP has been developed. Effects of glucose-6-phosphate and glycogen in the regulation of glycogen synthetase phosphatase have been characterized. A cyclic AMP-dependent protein kinase from rat liver has been partially purified and separated into subunits. An isolated rat liver preparation, an analogue model to glucose (catabolite) repression in bacteria, has been developed and partially characterized with regard to the action of glucose on cyclic AMP production. N-acetyltransferase has been characterized with regard to its pattern during development and its regulation by environmental lighting and neural pathways. Stimulation of the enzyme by norepinephrine has been shown to be mediated by a receptor which activates adenyl cyclase and increases cyclic AMP production. Substrate stabilization of the active form of the enzyme has been demonstrated.

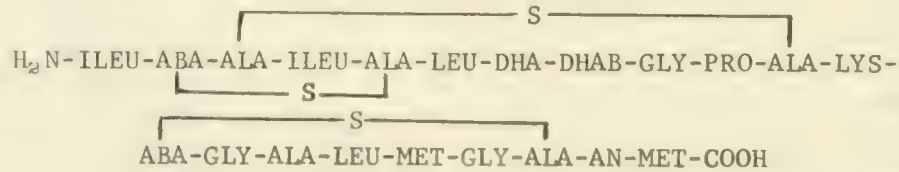
The Section on Developmental Pharmacology studies the subcellular processes involved in the induction of pharmacologically important enzymes by drugs, polycyclic hydrocarbons, or insecticides. These environmental stimuli induce the mixed-function oxygenases in maternal tissues, in the placenta, and transplacentally in fetal tissues. Consequently, we are concerned with (1) possible effects of these compounds on normal fetal growth and development, (2) mechanisms of "pharmacological immaturity" of the premature and newborn, (3) mechanisms of disease of the fetus and newborn concerning oxygenative and conjugative metabolism of normal body substrates such as steroids and bilirubin, and (4) possible therapeutic and diagnostic procedures for aiding and predicting pharmacogenetic defects in the fetus and neonate.

In fetal cell cultures derived from the entire hamster or mouse or from rat liver, we have developed an experimental model having numerous advantages over studies in the whole animal. Thus, we have established a chronological

sequence of events occurring during aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons: (1) passive intracellular entry and binding of inducer, (2) synthesis of induction-specific RNA, (3) translation of this RNA into induction-specific protein(s), (4) appearance of a high-spin CO-binding cytochrome associated with hydroxylase activity, and (5) enzymic metabolism of the inducer leading to covalent binding of some metabolites to cellular material and predominantly to excretion of metabolites from the cell. With cultures from certain mouse strains or from rat hepatoma in which hydroxylase induction does not occur, we obtained evidence that the defect is transcriptional. In mouse genetic studies the response of hydroxylase induction in liver, bowel, or kidney to systemic polycyclic hydrocarbons follows a Mendelian autosomal dominant pattern. The sequence of events occurring during oxygenase induction in fetal rat heaptocyte cultures by phenobarbital or insecticides is also under investigation. Nucleolar synthesis of ribosomal RNA may be of more importance in the process of hydroxylase induction by phenobarbital than that by polycyclic hydrocarbons. We have found that the primary action of phenobarbital, like that of polycyclic hydrocarbons, is at the level of transcription and that with either inducer there is also a secondary effect of lesser magnitude at the posttranslational level in which the normal rate of decay of induced enzyme is slowed.

The Section on Molecular Structure (1) develops fundamental methods for the structural elucidation and functional correlation of molecules of biological significance and (2) provides advanced approaches to the automation of laboratory processes. The Section isolates cell organelles, studies mechanisms of memory and learning, identifies the action at the cellular level of compounds with preselected structural elements, and establishes the resultant effect on intrauterine development. The more chemically oriented activities of the Section supplement other endeavors of the Laboratory whenever the need arises to determine the structure of constituents of biological systems.

Studies within the Section are progressing in these areas: (1) Approaches to the purification and characterization of nisin and of fragments of nisin have been improved considerably. Chromatography on Sephadex prior to countercurrent distribution is no longer a necessity. Extensive quantities of the peptide are more readily available for the investigation of induced fetal resorption. (2) The elucidation of the primary structure of nisin is complete with the exception of the assignment of two sulfide bridges and the clarification of a molecular rearrangement. The structure of the H_2N-terminal fragment of nisin follows.



(3) The synthesis of nisin has been initiated with the preparation of the COOH-terminal tripeptide VAL-DHA-LYS (valyldehydroalanyllysine). This peptide will subsequently be entered into studies on fetal resorption; it will also be used in the elucidation of the causes of the resistance of S-methylcysteine in the penultimate position of nisin to attack by cyanogen

bromides. (4) The structural elucidation of subtilin has been greatly facilitated with the successful fragmentation of the molecule by trypsin and thermolysin under specifically established conditions. This partial structure may presently be written for subtilin: H_2N-TRY-LY-LAN, β-CH_3-LAN_2, GLU_2, PRO, GLY_2, VAL, LEU_2, ALA-PHE, ASP, β-CH_2-LAN_2-GLU, LEU_2-LYS-ILEU-DHA-LYS-COOH. As with nisin, the presence of two residues of dehydroalanine and one residue of β-methyldehydroalanine is now also firmly established for subtilin. (5) The isolation of lysosomes has been further improved by the use of the cell disruption bomb which provides mild conditions for tissue homogenization. Protein constituents of the lysosomal membrane have been isolated and characterized by amino acid analysis. Indications are that the lysosomal membrane contains a protein (or proteins) of relatively low molecular weight--approximately 6000. (6) Automated sample application in amino acid analysis has been in use throughout the year and assures us of the virtually non-interrupted operation of one analyzer 24 hours a day. The construction of a second automated sample applicator is nearing completion and will be installed in the near future. (7) The solid phase synthesis of gramicidin B and of gramicidin C has been completed. Chemical and preliminary physical characterizations indicate identity with the native peptides. As was done with gramicidin A, the two peptides are to be entered into extensive biological tests. (8) Intraperitoneally administered nisin causes fetal resorption in the rat to the extent of 90%. The peptide appears to display this biological activity throughout the entire gestational period--the phenomenon more appropriately to be described as "inhibited implantation" for the early days of rat pregnancy. Intraperitoneal doses of nisin also induces fetal resorption in the rabbit. The peptide shows little or no effect on the rat conceptus when given orally. Fetal resorption may be induced by intraveneous administration of nisin--a drawback being the poor solubility of nisin in aqueous medium. Cyanogen bromide-produced fragments of nisin, though readily soluble in water, produce toxic effects in rodents. These compounds are presently being tested for their ability to induce fetal resorption.

Serial No. HD-LBC6

1. Laboratory of Biomedical Sciences
2. Section on Molecular Genetics
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Protein Synthesis: Mechanism and Role in the Regulation of Cell Growth

I. Initiation of Protein Synthesis
II. Mechanism of Polypeptide Chain Elongation
III. Genetic Analysis of the Protein Synthetic Apparatus
IV. Translational Control in Viral Gene Expression
V. Translational Control in Antibody Biosynthesis

Previous Serial Number: Same

Principal Investigator: P. Leder

Other Investigators:
A. Bernardi
R. Callahan
S. Krauss
H. Aviv
I. Boime
I. Schechter

Cooperating Units: None

Man Years

Total:	81/12
Professional:	69/12
Others:	12/12

Project Description:

Objectives: To define in genetic and molecular terms the mechanisms which initiate protein synthesis, allow formation of initial and subsequent peptide bonds, and provide for the orderly translocation of mRNA across the ribosome during translation. Further, to understand the regulatory mechanisms which control gene expression at the level of translation in normal as well as in both bacteriophage and animal virus-infected cells.

Methods Employed: Cell-free protein synthetic systems are derived from both Escherichia coli, and the Krebs II ascites tumor cell. Elements involved in the synthesis of proteins in these systems are purified using conventional procedures. Authentic polycistonic mRNA's are derived from several small RNA bacteriophages and from the two animal viruses, encephalomyocarditis (EMC) and polio. Additional mRNA species corresponding to specific enzymes are synthesized in vitro using purified viral DNA and specific viral RNA

polymerase. In addition to the physical, chemical, and biologic methods employed to purify and assemble these protein synthetic systems, genetic techniques are used to obtain and map mutations which effect the protein synthetic elements and control processes regulating the translation of mRNA. Additional aspects of the study require the preparation of specific antibodies to these protein synthetic elements as well as the growth of tumor cells, both in culture and in vivo.

Major Findings: (1) A purified cell-free system previously devised in this laboratory was used to detect specific initiation sites on viral, F2, Qβ, and T7 polycistonic mRNA's. (2) The system was shown to initiate and to translate accurately, permitting the identification of the initial peptides of two Qβ bacteriophage proteins. These were correlated with specific genes, the Qβ coat and the Qβ RNA-polymerase. (3) Using this system, a specific translational repressor, the Qβ coat protein, was found to prevent initiation of the Qβ polymerase cistron, thus isolating and identifying its site of action. (4) Further studies revealed the initial amino acid sequence of the polymerase protein and permitted the prediction of the nucleotide sequence of its corresponding mRNA, the region affected by the repressor. (5) Genetic and biochemical studies resulted in the characterization of an E. coli mutant effected in its ability to translocate (ribosomal movement with respect to mRNA). (6) Our prediction that such a mutation should be closely linked to other markers effecting ribosomal function was verified by genetic transduction and, further, showed that this function was closely linked to an element involved in the regulation of messenger RNA synthesis, the cyclic-AMP binding protein.

Significance to Biomedical Research and the Program of the Institute: Cellular control mechanisms obviously effect the sequential expression of genetic information during the course of an organism's development. Our understanding of these mechanisms in model systems provides a basis for their understanding in man at several levels of organization. The viral control studies are particularly relevant to disorders which may be induced by a viral-host interaction, such as birth defects.

Proposed Course of Project: Details of the bacteriophage regulatory system and the genetic locus controlling the protein synthetic apparatus will be further elucidated. When the nucleotide sequence of the controlled region of the phage cistron is known, an effort will be made, using synthetic nucleotide sequences, to interfere with this control mechanism and thus provide a basis for the generation of anti-viral compounds. Work with the protein synthesis has already been extended to a mammalian system which accurately translates viral mRNA's. This system will be used to study viral control of host protein synthesis. It will also be used as a tool to detect mRNA's corresponding to specific γ-globulins in an effort to use immunogenesis as a model in the study of induced protein synthesis in higher organisms.

Honors and Awards: None

Publications:

1. Skogerson, L., Roufa, D., and Leder, P.: Characterization of the initial peptide of Qβ RNA polymerase and control of its synthesis. Proc. Nat. Acad. Sci., U.S.A. 68: 276-279, 1971.

2. Bernardi, A., and Leder, P.: Protein biosynthesis in Escherichia coli. Purification and characteristics of a mutant G factor. J. Biol. Chem. 245: 4263-4268, 1970.

3. Leder, P.: The genetic code. In Sober, H. (Ed.): The Handbook of Biochemistry. Cleveland, Ohio, The Chemical Rubber Co., 1970, p. I90.

4. Leder, P., Roufa, D., Skogerson, L., and Bernardi, A.: Protein biosynthesis: Studies using synthetic and viral mRNA's. Eighth International Congress of Biochemistry, Proceedings, p. 202, 1970.

5. Roufa, D. J., Doctor, B. P., and Leder, P.: The two site model of ribosomal function: A test using degenerate serine codons in bacteriophage f_2 mRNA. Biochem. Biophys. Res. Commun. 39: 231-237, 1970.

6. Roufa, D. J., Skogerson, L. E., and Leder, P.: Translation of phage Qβ mRNA: A test of the two-site model for ribosomal function. Nature 227: 567-570, 1970.

7. Leder, P., Bernardi, A., Livingston, D., Loyd, B., Roufa, D., and Skogerson, L.: Protein biosynthesis: Studies using synthetic and viral mRNAs. Cold Spring Harbor Symposia on Quantitative Biology XXXIV: 411-417, 1969.

8. Leder, P.: Preparation of homogeneous E. coli translocation factor G. In Colowick, S. P., and Kaplan, N. O. (Eds.): Methods in Enzymology. New York, N. Y., Academic Press, Volume 20, in press.

9. Roufa, D. J., and Leder, P.: Biosynthesis of phage-specific initiation dipeptides; a purified biosynthetic system derived from E. coli. J. Biol. Chem., in press.

Serial No. HD-LB16

1. Laboratory of Biomedical Sciences
2. Section on Molecular Genetics
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Control Mechanisms in Temperate Bacteriophage
I. The Control of Normal Phage Integration and Excision
II. Secondary Pathways of Phage Integration and Excision
III. The Physiology of Abnormally Excised Phage

Previous Serial Number: Same

Principal Investigator: R. Weisberg

Other Investigators: K. Shimada

Cooperating Units: Laboratory of Molecular Biology, Section on Biochemical Genetics, NCI
Division of Biology, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Man Years

Total:	24/12
Professional:	24/12
Others:	0

Project Description:

Objectives: To define the factors necessary for phage integration and excision and their interaction with one another and with the phage chromosome; to understand the control the phage exerts over the synthesis of these factors; to study the nature of abnormal phage integration and excision with the aims of understanding the normal process, on the one hand, and of determining the feasibility of using abnormal integration as a tool for biochemical analysis of bacterial gene products, on the other.

Methods Employed: The organisms of study are bacteriophage λ, other genetically related phages, and their host, the bacterium E. coli. Genetic techniques for the selection of lysogenic cells are being used to select phages integrated at abnormal host chromosomal sites; phage mutants with specific chromosomal aberrations are being used to characterize the activity and stability of the factors which govern integration and excision. Temperature sensitive control mutants are being used to study the regulation of the synthesis of these factors.

Major Findings: (1) Normally, phage λ integrates into the bacterial chromosome at a specific chromosomal site. We have found that integration still occurs,

although at reduced frequency, in mutant bacteria which have lost this specific site. Many new sites of phage integration have thus been identified. Integration into and excision from these abnormal sites mimics normal integration in that the same enzymatic machinery (the products of the phage int and xis genes) and the same site on the phage chromosome are employed. Occasionally, insertion occurs within a known bacterial gene, thus inactivating gene function and producing a mutant lysogen. By means of appropriate mutant selection techniques we have been able to direct integration within chosen genes. Novel types of transducing phages (phage particles which carry portions of the bacterial chromosome) have been isolated from bacteria carrying phage λ at abnormal chromosomal sites. (2) We have continued our studies (see last year's report) on the in vivo instability of the phage xis gene product. (Xis-gene function is required for excision of the integrated phage chromosome from the bacterial chromosome; int-gene function is required both for integration and excision.) Since phage integration is an irreversible event in the absence of the xis gene product, its instability helps to explain why λ integration is essentially 100% efficient. Decay of xis product in the cell is an active process--it can be blocked by metabolic inhibitors. (3) Three different and apparently independent enzyme systems are known to promote genetic recombination in phage λ. They are: (a) the int pathway (phage controlled); (b) the red pathway (also phage controlled); and (c) the rec pathway (specified by bacterial genes). We have found that all three pathways can produce recombinants in which the molecules of the parental chromosomes have been conserved. Thus, neither int-, red-, nor rec-promoted recombination need involve complete de novo synthesis of recombinant chromosomes. Instead, it is more likely that recombinants are produced by enzymatically produced breakage, exchange, and rejoining of preexisting parental chromosomes. (4) We have found that the expression of the λ int and xis genes is directly repressed by another phage protein, the repressor. Expression of genes int and xis in a lysogen does not occur unless repressor function is inactivated and, in addition, the phage synthesize an activator (the product of gene N). If either of these two conditions is not met, transcription of genes int and xis does not occur.

Significance to Biomedical Research and the Program of the Institute: The integration and excision of viral (and other episomal) DNA is known to change the physiology of the host organism. In addition, these viruses (and other episomal elements) provide an important mechanism of genetic transfer and exchange in the microbial world. It is highly likely that such a successful symbiotic relationship has an analogy in the relationship between certain animal viruses and the cells of higher organisms. Moreover, an understanding of the control mechanisms operative in these easily studied microorganisms has obvious relevance to an understanding of regulatory elements in higher organisms. The ability to direct phage integration to predetermined locations on the host chromosome should prove to be an extremely useful and widely applicable method for isolating bacterial gene products and studying the control of bacterial gene expression.

Proposed Course of Project: We intend to further characterize the mechanism of phage integration into abnormal sites and its relation to normal integration. We hope to construct new transducing phages for regions of the

bacterial chromosome that are of particular interest. We are continuing our study of the mechanism of decay of the xis gene product.

Honors and Awards: None

Publications:

1. Weisberg, R.: Requirements for curing of λ lysogens. Virology 41: 195-199, 1970.

2. Weisberg, R., and Gottesman, M.: The stability of Int and Xis functions. In Hershey, A. D. (Ed.): The Bacteriophage λ. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory of Quantitative Biology, in press.

3. Gottesman, M., and Weisberg, R.: Prophage insertion and excision. In Hershey, A. D. (Ed.): The Bacteriophage λ. Chapter 6. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory of Quantitative Biology, in press.

4. Kellenberger-Gujer, G., and Weisberg, R.: Recombination in bacteriophage λ. I. Material exchange of DNA during Int-, Red- or Rec-promoted recombination. In Hershey, A. D. (Ed.): The Bacteriophage λ. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory of Quantitative Biology, in press.

Serial No. HD-LB20

1. Laboratory of Biomedical Sciences
2. Section on Molecular Genetics
3. Bethesda

PHS-NIH
Individual Project Report
October 5, 1970 through June 30, 1971

Project Title: Regulation of Antibody Biosynthesis
I. Mechanisms of consignment of cells to determinant-specific immunocompetency
II. Deviation of immunocompetent cells

Previous Serial Number: None

Principal Investigator: E. E. Hanna

Other Investigators: A. Uzunova

Cooperating Units: None

Man Years

Total:	18/12
Professional:	18/12
Others:	0

Project Description:

Objectives: (1) To discover and map cellular pathways of antibody biosynthesis as defined by secretion of antigen binding immunoglobulin after cell contact with antigen or antigen information; (2) to define the role of macrophages in the consignment of lymphoid (secreter) cells to induced monospecific immunoglobulin secretion; (3) to devise means of selectively regulating synthesis and secretion of specific antibodies by cells. Selective stimulatory action by antigen determinants receive emphasis in this scheme.

Methods Employed: Immunogens chemically substituted with small defined chemical groups (haptens) are prepared. Rabbits and mice as well as isolated lymphoid cell cultures are immunized and the resulting immune cell populations recovered. Cell separation techniques such as differential attachment and recovery of cells from plastics, particles, and glass, and density gradient elutions allow for cell isolations. A cryogenic apparatus allows rate-freezing of cells for storage and recovery of functional cells. Activity and function of cells are quantitated using unique and simple but effective modifications of the localized hemolysis-in-gel (LHG) method. These allow enumeration and analysis of cells secreting antibodies to defined small haptens (hapten-specific LHG). Further adaptations allow study of population dynamics (replica hapten specific LHG).

Major Findings: We have found that immunocompetent cells secrete intact immunoglobulin molecules. The biologic activity is seen directly as discrete zones of red cell lysis in the immediate vicinity of single cells. The activity is shown to be directed specifically at the substituted chemical groups of the immunogen used to stimulate proliferation of the secreting cells.

A strong competition between monospecific cell populations is observed when an immunogen bearing a mixture of determinants is presented. Proliferation of one monospecific cell population at the expense of a competing population may be observed after changes in determinant ratios of the carrier molecule or particle. These observations suggest that finite antigen determinants are stimuli of specific cellular proliferation and may exert selective pressures upon heterogeneous populations at monospecific cells.

Significance to Biomedical Research and the Program of the Institute: The ability to regulate selectively and specifically the immune response will have great advantage over current measures which employ hazardous generalized immunosuppression and stimulation. The results should enhance and complement scientific thought on cellular control mechanisms in general.

Proposed Course of Project: Essential proof or substantiation of the postulated mechanism set forth above will be sought using cell culture and cell separation techniques.

Positive results should allow assignment or mapping of functional roles within pathways of cellular biosynthesis of antibodies.

It will be of particular interest to define the role(s) of the macrophage(s) in light of the fact that an information transfer role has been strongly suggested but not substantiated.

Honors and Awards: None

Publications:

1. Hanna, E. E., and Merchant, B.: Secretion of monospecific anti-hapten IgG antibodies by rabbit immunocytes. J. Immun., in press.

Serial No. HD-LB1(c)

1. Laboratory of Biomedical Sciences
2. Section on Developmental Enzymology
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Developmental Enzymology

Previous Serial Number: Same

Principal Investigator: J. C. Robinson

Other Investigators: J. B. Edlow
J. F. Huddleston
G. Lee

Cooperating Units: Department of Obstetrics and Gynecology, Washington Hospital Center, Washington, D. C.

Man Years

Total:	33/12
Professional:	22/12
Others:	11/12

Project Description:

Objectives: The main objectives of this project are: (1) to study qualitative and quantitative changes in selected enzyme systems in the developing placenta and fetal organs in normal gestation; (2) to study qualitative and quantitative changes in these same enzyme systems in the developing placenta and fetal organs in reproductive disorders such as abortions, stillbirths, and fetal malformations; (3) to isolate, purify and characterize these enzymes from the normal placenta; and (4) to identify and characterize pregnancy-associated enzymes in maternal blood plasma.

Methods Employed: The principal methods employed are: (1) the zymogram technique, by which electrophoretic variants of enzymes can be detected; (2) autoradiography, with which potential carrier function is evaluated (for example, by using I^{131}-tagged thyroxine); (3) enzyme assays, which are usually based on spectrophotometric methods; (4) protein isolation techniques, including salt- and solvent-fractionation, gel-filtration, and ion-exchange chromatography; and (5) immunochemical procedures.

Major Findings: (1) Optimal conditions were established for the preparation of zymograms of three placental enzymes: glucose dehydrogenase, β-glucuronidase, and N-acetyl-β-glucosaminidase. (2) One of these enzymes, glucose dehydrogenase, was purified 5000-fold by a sequence of ammonium sulfate

fractionation, DEAE chromatography, and Sephadex gel filtration. (3) β-Glucuronidase and N-acetyl-β-glucosaminidase have been partially purified. Two major isoenzymic fractions of N-acetyl-β-glucosaminidase have been separated by adsorption techniques. (4) Six placental enzymes were studied throughout gestation for developmental changes in isoenzyme patterns and total activities. Of these 6, only lactic dehydrogenase showed a change in zymogram pattern. However, 3 enzymes, N-acetyl-β-glucosaminidase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase, exhibited significant changes in total activity. (5) Striking changes in isoenzyme patterns and in total enzyme activities were observed in two placentas associated with "blighted" ovum abortions.

Significance to Biomedical Research and the Program of the Institute: The study of placental enzymes in normal pregnancy will provide a suitable base-line with which one can compare the same enzyme systems in reproductive disorders such as stillbirths and abortions. For example, consistent changes in enzymes in abortion material may reflect differences in placental function, which in turn could affect fetal viability. Hopefully, these studies will lead to increased understanding of both normal and abnormal biochemical processes involved in pregnancy and fetal development.

Proposed Course of Project: (1) Characterization of selected placental enzymes and enzymes in maternal plasma and in amniotic fluid will be continued. (2) Correlations between abnormal pregnancies and variations in fetal or maternal enzyme systems will be sought.

Honors and Awards: None

Publications:

1. Volpe, J. J., Lee, G., Laster, L., and Robinson, J. C.: Regional distribution of isozymes of D-amino acid oxidase and acetylesterase in developing primate brain. Exp. Neurol. 28: 76-87, 1970.

2. Huddleston, J. F., Lee, G., and Robinson, J. C.: Electrophoretic characterization of glucose dehydrogenase, β-glucuronidase, and N-acetyl-β-glucosaminidase from the human placenta. Amer. J. Obstet. Gynec. 109: 1017-1022, 1971.

Serial No. HD-LB2

1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Intracellular Proteolytic Enzymes

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: W. Gutensohn
K. Bromwell

Cooperating Units: None

Man Years

Total:	14/12
Professional:	8/12
Others:	6/12

Project Description:

Objectives: The objectives of this research are: (1) to purify and characterize specific intracellular proteolytic enzymes; (2) to determine the function of these enzymes; and (3) to investigate how these enzymes are linked into the overall metabolism of the cell.

Methods Employed: The methods used include standard techniques of protein purification and assay as well as fluorometric analysis.

Major Findings: (1) Observation and purification of an inhibitor for an intracellular proteinase in the cell supernatant fraction.

Significance to Biomedical Research and the Program of the Institute: Although intracellular proteolytic enzymes have been recognized for many years, their function is unknown. Assignment of a mechanism of action, physiological or enzymatic, to one of these enzymes would add significantly to biochemical information. Such information might also be useful therapeutically.

Proposed Course of Project: (1) Complete purification of the proteolytic inhibitor; (2) characterization as to properties, specificity, and mode of action; (3) attempts to produce homologous antibodies and observe what effect this has on the animal.

Honors and Awards: None
Publications: NONE

Serial No. HD-LB9

1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Models of Mental Retardation

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: A. Andersen
P. Rosenblitt

Cooperating Units: None

Man Years

Total:	14/12
Professional:	14/12
Others:	0

Project Description:

Objectives: The objectives of this research are: (1) to produce biochemical models of mental retardation syndromes in laboratory animals by chemical means; (2) to investigate the pathological and behavioral aspects of the chemical treatments.

Methods Employed: The methods employed include standard enzymatic assay techniques, histological examinations, and behavioral evaluation.

Major Findings: (1) p-Chlorophenylalanine inhibits rat liver phenylalanine hydroxylase irreversibly and, together with phenylalanine, produces a condition biochemically resembling phenylketonuria. (2) α-Methyl-p-chlorophenylalanine also inhibits phenylalanine hydroxylase. (3) p-Chlorophenylalanine lowers siezure threshhold in young animals. (4) Treatment of newborn rats with p-chlorophenylalanine and phenylalanine produces an irreversible deficit in the adult rat's performance in open field, avoidance conditioning, and maze performance, as well as a slightly decreased brain weight.

Significance to Biomedical Research and the Program of the Institute: No adequate model of mental retardation has yet been produced in a laboratory animal. The need for such a disease analog is great, since only then can investigations be initiated toward understanding the biochemical basis for the retardation. The endeavor to produce a chemical model in this Laboratory centers around phenylketonuria because it is the best characterized from a biochemical and a behavioral aspect and because a great deal is known about

the enzyme which is deleted, phenylalanine hydroxylase. If such a model can be produced, substantial information should be available about the causes and perhaps the prevention of the retardation.

Proposed Course of Project: (1) A complete biochemical profile of the effects of p-chlorophenylalanine, a potent phenylalanine hydroxylase inhibitor; (2) studies on its mode of inhibition; (3) administration of this drug to rats during development and pathological and behavioral evaluation after growth to maturity; (4) attempts to prevent the defect from developing by simultaneous treatment with other materials; (5) studies of the appearance of associated brain lesions; (6) expansion of this approach to a consideration of other metabolic abnormalities leading to retardation.

Honors and Awards: None

Publications:

1. Prichard, J. W., and Guroff, G.: Increased cerebral excitability caused by p-chlorophenylalanine in young rats. J. Neurochem. 18: 153-160, 1971.

Serial No. HD-LB10

1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mechanism of Aromatic Hydroxylation

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: J. Dayman
G. Dickens

Cooperating Units: None

Man Years:

Total:	15/12
Professional:	6/12
Others:	9/12

Project Description:

Objectives: The objectives of this research are: (1) to provide information on the process of enzymatic hydroxylation; and (2) to get detailed information on the nature of phenylalanine hydroxylase.

Methods Employed: The methods employed include standard techniques of enzyme kinetics and purification, antibody detection, and organic synthesis and characterization.

Major Findings: (1) Serum albumin influences the hydroxylase reaction by binding the iron cofactor and this may represent a general mechanism for albumin effects which have been observed with many hydroxylases. (2) Antibodies to bacterial phenylalanine hydroxylase can be produced in sheep but these antibodies do not cross-react with the phenylalanine hydroxylase from rat liver.

Significance to Biomedical Research and the Program of the Institute: Information about phenylalanine hydroxylase and about ways to inhibit or stimulate it relates directly to the problem of mental retardation since the enzyme in question is the one missing in the best characterized of the genetic diseases, phenylketonuria. Detailed analysis of the structure of this enzyme should provide new and unique information since very little has been accomplished on hydroxylases in general and no pteridine enzymes have been reported as homogeneous preparations.

Proposed Course of Project: (1) Complete purification of Comamonas phenylalanine hydroxylase; (2) study of the action of the enzyme on a number of substrate inhibitors in an attempt to determine the nature of the intermediates in the hydroxylation process; (3) detailed study of pteridine cofactor requirement with especial reference to alterations in the side chain.

Honors and Awards: None

Publications:

1. Guroff, G.: Phenylalanine hydroxylase from Pseudomonas sp. In Tabor, H., and Tabor, C. W. (Eds.): Methods in Enzymology. New York, Academic Press, 1970, Vol. XVII A, pp. 597-603.

2. Guroff, G., Karadbil, M., and Dayman, J.: The effect of bovine serum albumin on the measurement of the activity of highly purified Comamonas phenylalanine hydroxylase. Arch. Biochem. 141: 342-345, 1970.

3. Guroff, G.: The hydroxylation-induced migration of tritium: Practical aspects. Atomlight 69: 1-5, 1969.

Serial No. HD-LB11

1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biosynthesis and Function of Pteridines

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: J. Dayman

Cooperating Units: None

Man Years

Total:	11/12
Professional:	11/12
Others:	0

Project Description:

Objectives: The objectives of this research are: (1) to provide information on the enzymes in the biosynthetic pathway to the pteridine ring; (2) to investigate the functions of pteridines in addition to their action as hydroxylase cofactors.

Methods Employed: The methods used include standard techniques of enzyme purification, isotope measurement, high-voltage electrophoresis, and fluorescence detection.

Major Findings: (1) Purification and study of the first enzyme in pteridine biosynthesis from Comamonas and characterization of its product as a new material, namely, a cyclic phosphate derivative of neopterin; (2) discovery and study of two enzymes, cyclic phosphate diesterases, which act on the cyclic derivative.

Significance to Biomedical Research and the Program of the Institute: The pteridine cofactors are important in a number of hydroxylase reactions including those leading to the neurohormones serotonin and norepinephrine. Information about the pharmacology and biochemistry of these biosynthetic enzymes may lead to the ability to inhibit or stimulate these pathways and perhaps to new therapeutic tools for maladies involving these neurohormones.

Proposed Course of Project: (1) Purification and characterization of the enzymes of pteridine biosynthesis in Comamonas; (2) investigation of the action of pteridine inhibitors on these enzymes; (3) attempts to link

pteridines or pteridine derivatives covalently to the enzymes they serve; (4) study of pteridine biosynthetic enzymes in photosynthetic organisms; (5) search for other functions of pteridines.

Honors and Awards: None

Publications:

1. Guroff, G.: Radioisotope assay for reduced pteridines: The phenylalanine hydroxylase cofactor. In McCormick, D. B., and Wright, L. D. (Eds.): Methods in Enzymology. New York, Academic Press, Vol. 18B, in press.

2. Cone, J., and Guroff, G.: Partial purification and properties of guanosine triphosphate cyclohydrolase, the first enzyme in pteridine biosynthesis, from Comamonas sp. (ATCC 11299a). J. Biol. Chem. 246: 979-985, 1971.

Serial No. HD-LB12

1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemistry of Brain Macromolecules

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: M. Brodsky
J. Ende

Cooperating Units: None

Man Years

Total:	17/12
Professional:	2/12
Others:	15/12

Project Description

Objectives: The objectives of this research are: (1) to investigate the biochemistry of brain macromolecules; (2) to determine if any aspects exist that are unique to brain; and (3) to find out whether any unique aspects are related to the unique function of the brain.

Methods Employed: The methods involved in this study include isotope techniques, high voltage electrophoresis, and tissue slice metabolic studies, as well as standard methods for purification and investigation of macromolecules.

Major Findings: (1) Rat brain uses uridine rather than orotic acid as a preferential source of pyrimidines. By implication this means that de novo synthesis of pyrimidines is less important in brain than in other organs. (2) Brain slices from young rats synthesize RNA more rapidly from several precursors than do comparable slices from adult animals. In young brain the RNA biosynthetic enzymes are present in slightly higher amounts and the degradative enzymes in much lower amounts. (3) Guanine-hypoxanthine:PRPP pyrophosphorylase and several adenine:PRPP pyrophosphorylases have been observed and studied in rat brain.

Significance to Biomedical Research and the Program of the Institute: Substantial evidence exists that brain macromolecules are involved in the unique functions of the brain. A continuing question exists as to the directness of this involvement. These studies may shed light on this question

by finding properties of brain macromolecules compatible with a unique function such as the storage of memory. Further, development of such systems as the tissue slice for RNA synthesis may provide tools for the investigation of the biochemical alterations occurring in certain types of mental retardation.

Proposed Course of Project: (1) Investigation of the purine:PRPP pyrophosphorylases in brain; (2) study the metabolic function of these enzymes using specific inhibitors; (3) characterization of the rapidly labeled RNA of rat brain slices.

Honors and Awards: None

Publications:

1. Hogans, A., Guroff, G., and Udenfriend, S.: Studies on the origin of pyrimidines for neural RNA biosynthesis in the rat. J. Neurochem., in press.

2. Guroff, G., and Brodsky, M.: Enzymes of nucleic acid metabolism in the brains of young and adult rats. J. Neurochem., in press.

3. Guroff, G.: Biochemistry of the brain. In Mason, H. S. (Ed.): Functional Biochemistry. MacMillan Co., in press.

4. Guroff, G.: Special aspects of amino acid metabolism. In Siegel, G. (Ed.): Basic Neurochemistry. Little, Brown and Co., in press.

Serial No. HD-LB13

1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mechanism of Amino Acid Transport

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: H. Kuzuya
K. Bromwell

Cooperating Units: None

Man Years

Total:	14/12
Professional:	8/12
Others:	6/12

Project Description:

Objectives: The objectives of this research are: (1) to describe the process by which amino acids enter cells; (2) to investigate biochemical and pharmacological effects on the transport system; and (3) to dissect the transport system.

Methods Employed: The methods employed include standard protein purification, isotope techniques, and bacterial culture operations.

Major Findings: (1) Complete purification and partial characterization of a phenylalanine-binding protein from transport-induced bacteria; (2) initial chemical investigations on the binding site which indicate the participation of lysine in the binding of phenylalanine to its carrier protein.

Significance to Biomedical Research and the Program of the Institute: The active transport of amino acids is a general property of cells. The transport of amino acids into brain or kidney, etc., may be a limiting or at least a contributory factor in their further metabolism. An understanding of the mechanisms of transport may provide insight into the normal functioning of cells and into ways to cope with transport malfunctions which occur in certain disease states.

Proposed Course of Project: (1) Study of the chemistry of the phenylalanine binding site on this protein; (2) search for comparable sites in transport systems of mammalian tissues.

Honors and Awards: None

Publications:

1. Guroff, G., and Bromwell, K. E.: Phenylalanine uptake and phenylalanine binding material in Comamonas sp. (ATCC 11299a). Arch. Biochem. 137: 379-387, 1970.

2. Kuzuya, H., Bromwell, K., and Guroff, G.: The phenylalanine binding protein of Comamonas sp. (ATCC 11299a). J. Biol. Chem., in press.

Serial No. HD-LB4

1. Laboratory of Biomedical Sciences
2. Section on Physiological Controls
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Regulation of Liver Metabolism

Previous Serial Number: Same

Principal Investigator: W. H. Glinsmann

Other Investigators: P. Sherline

Cooperating Units: G. Pauk, Department of Internal Medicine, University of Texas Medical School, Galveston, Texas

Man Years

Total:	33/12
Professional:	15/12
Others:	18/12

Project Description:

Objectives: The general objective is to clarify the mechanisms involved in hormonal and nutrient control of hepatic carbohydrate metabolism and protein synthesis. The current focus is on the detailed description of reaction mechanisms modulating interactions between glucose, amino acids, and hormones which act by altering the production of 3',5'-cyclic nucleotides.

Methods Employed: Physiological and pharmacological experiments were performed using rats and an isolated rat liver perfusion system. Chemical and enzymatic assays in conjunction with these experiments were performed on serum, perfusate, liver homogenates, and subcellular or protein fractions of liver tissue. Specific enzymes and cyclic nucleotide binding proteins were partially purified and were analyzed regarding the effects of metabolic intermediates on their kinetic behavior.

Major Findings: 1. Regulation of rat liver phosphorylase and glycogen synthetase activities by glucose and cyclic AMP: The role of hyperglycemia in the activation of glycogen synthetase and the inactivation of phosphorylase in the isolated perfused rat liver were further examined. An isotope displacement assay for cyclic AMP (developed by A. Gilman, NIH) was modified to measure routinely and rapidly as little as 0.03 picomoles of cyclic AMP in biological materials. It is based on the binding of the nucleotide to a partially purified muscle protein kinase preparation. With this assay it has been shown that glucose can markedly effect a net dephosphorylation of

phosphorylase and glycogen synthetase without altering tissue levels of cyclic AMP or production rates as estimated from rates of hepatic nucleotide efflux. In this regard, the effect of glucose in rat liver is dissimilar to that noted in bacterial systems where its action to regulate metabolism and enzyme induction is secondary to lowered intracellular levels of cyclic AMP.

Inhibitors of α- and β-adrenergic receptor sites do not modify the action of glucose on liver phosphorylase or glycogen synthetase. This finding supports the idea that the effect of glucose is not mediated by alterations in the release or action of biogenic amines.

Insulin regulation of hepatic glycogenolysis via alterations in the activities of phosphorylase and glycogen synthetase appears to be mediated by its action to lower intracellular levels of cyclic AMP. The observation that insulin inhibits the action of glucagon-induced increase in intracellular levels of cyclic AMP has been confirmed and correlated with alterations in the activities of these enzymes. Lack of correlation of the effects of insulin on proteolysis, amino acid oxidation, and K^+ flux with alterations in tissue levels of this nucleotide support the hypothesis that regulation of these processes by insulin is not secondary to changes in rates of cyclic AMP production.

Our previous studies on the control of synthesis of liver glycogen synthetase phosphatase supported the hypothesis that this enzyme (necessary for expression of an action of glucose in the dephosphorylation of glycogen synthetase) required insulin. Additional <u>in vivo</u> studies in the rat with diazoxide, an inhibitor of pancreatic insulin release, support this view.

A possible acute regulation of liver glycogen synthetase phosphatase activity by glucose metabolites was investigated. Glucose-6-phosphate and Mg^{++} activate this phosphatase in desalted protein fractions. Glycogen was inhibitory to this activation. Purification of this phosphatase and glycogen synthetase substrate is being conducted to further examine this regulatory mechanism.

The primary step in the action of cyclic AMP to phosphorylate glycogen enzymes (and possibly regulate transcriptional and translational steps in protein synthesis) involves the activation of a protein kinase. Rat liver protein kinase and cyclic AMP binding protein fractions have been purified 100-200 fold. To date we have been unable to demonstrate any effect of glucose or glucose metabolites on the activity of the kinase, the requirements of the kinase for ATP or Mg^{++}, or the binding affinity or total number of binding sites for cyclic AMP. Further studies in this area will be conducted following the separation of protein kinase subunits and the purification of enzyme (glycogen synthetase-a, phosphorylase-b kinase) substrates for the protein kinase.

2. <u>Regulation of rat liver phosphorylase by α-adrenergic receptors</u>: It has been proposed that α-adrenergic receptors mediate a fall in tissue levels of cyclic AMP and β receptors mediate a stimulation of adenyl cyclase and hence a rise in tissue cyclic AMP. Studies in the isolated perfused rat liver

support this mechanism and further support the hypothesis that α-adrenergic stimulation activates phosphorylase by a mechanism which does not involve increased production of cyclic AMP.

3. Glucose inhibition of tyrosine aminotransferase induction: In the isolated rat liver, the induction of tyrosine aminotransferase by amino acids is markedly inhibited by hyperglycemia, and this inhibition is rapidly reversed by glucagon and cyclic AMP. This model, which appears analagous to catabolite repression seen in bacterial systems, differs in that inhibition of this induction is not associated with lowered tissue levels of cyclic AMP.

Significance to Biomedical Research and the Program of the Institute: Regulation of cellular metabolism, growth, and differentiation at many phylogenetic levels appears to be mediated by a complex series of interactions between glucose and nutrients and factors which alter the net production or cellular levels of cyclic AMP. In mammalian systems, many polypeptide hormones and biogenic amines act primarily by altering the formation of this nucleotide. Examination of the mechanisms by which preexisting nutritional state and acute alterations in nutrient supply modify these hormonal responses in liver will hopefully increase our understanding of factors which regulate the development and adaptations of hepatic enzyme systems and control glucose homeostasis. There is now ample evidence that the regulatory systems on which this project has focused are common to many animal species and are also involved in the regulation of protein and DNA synthesis and cellular growth.

Proposed Course of Project: Studies will continue to explore mechanisms which control cyclic nucleotide, protein, and carbohydrate metabolism in liver.

Honors and Awards: None

Publications:

1. Berg, G., and Glinsmann, W. H.: Cyclic AMP in depression and mania. Lancet 1, 834, 1970.

2. Glinsmann, W. H., Pauk, G., and Hern, E.: Control of rat liver glycogen synthetase and phosphorylase activities by glucose. Biochem. Biophys. Res. Commun. 39: 774-782, 1970.

3. Linarelli, L. G., and Farese, R. V.: The effects of imidazole on adrenal metabolism in vitro. Pharmacology 4: 33-38, 1970.

Serial No. HD-LB18

1. Laboratory of Biomedical Sciences
2. Section on Physiological Controls
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Regulation of Neuroendocrine Metabolism

Previous Serial Number: Same

Principal Investigator: D. C. Klein

Other Investigators: G. R. Berg

Cooperating Units:
S. Strada, and B. Weiss, Laboratory of Preclinical Pharmacology, Division of Special Mental Health Research, NIMH
R. Reiter, Department of Anatomy, University of Rochester, Rochester, New York
R. E. Moore, Division of Neurology, University of Chicago, Chicago, Illinois
R. Quarles, Laboratory of Neurochemistry, NINDS
I. T. Lott, Children's Diagnostic and Study Branch, NICHD
H. Lynch and C. L. Ralph, Department of Biology, University of Pittsburgh, Pittsburgh, Pennsylvania

Man Years

Total:	50/12
Professional:	32/12
Others:	18/12

Project Description:

Objectives: A primary objective of this project is the description of the factors and mechanisms which are important in the regulation of melatonin production in the pineal gland including environmental influences, neural pathways, and endocrine controls. A second objective is the description of factors which govern the appearance during development of enzymes in the melatonin pathway in the pineal gland. Factors which control the development of the innervation of the pineal gland are also being determined.

Methods Employed: The physiological regulation of melatonin metabolism is studied by measuring the concentration of melatonin in the pineal gland and the activity of specific enzymes in the serotonin → melatonin pathway. The importance of specific endocrine organs and neural structures to this regulation is determined by ablation and neural-section experiments. The

development of enzyme systems is studied by correlating their time of appearance with other factors in the pineal gland which may regulate development.

The molecular mechanisms involved in the physiological regulation of metabolism and development are studied in an *in vitro* system. Pineal glands from rats of different ages are cultured in highly defined conditions; and the effect of potential regulators, such as hormones and neurotransmitters, are examined by measuring the activity of enzymes and the appearance of specific products following treatment. The mechanisms through which these regulators act on enzymes and products has also been examined. This system has also been used to study the production and secretion of glycoproteins that are radiolabelled with ^{3}H-fucose, as described in HD-CD38.

Major Findings: 1. Regulation of pineal N-acetyltransferase: A 15-60 fold nocturnal increase in the activity of N-acetyltransferase occurs in the adult rat pineal gland. In 15 other tissues examined, a nocturnal increase greater than 2 fold was not observed. The increase in pineal glands is caused by removal of lighting. The suppressive effect of lighting is mediated by the eye and the superior cervical ganglia. The nocturnal increase is independent of the gonads and the Harderian glands but appears to depend on the pituitary. The activity of pineal N-acetyltransferase at night disappears with an apparent half-life of 3 minutes, when the lights are turned on. Following a return to low enzyme value, enzyme activity can be stimulated by darkness only after 14 hours of lighting.

In vitro studies indicate that norepinephrine regulates pineal N-acetyltransferase via a cyclic AMP mechanism which depends upon protein synthesis. Only similar catechol amines and dibutyryl adenosine 3',5'-monophosphate have a similar effect. Indole amines are ineffective. Norepinephrine stimulates pineal adenyl cyclase, cyclic AMP, N-acetyltransferase, and melatonin production through a beta adrenergic receptor system.

The activity of N-acetyltransferase in homogenates of pineal glands obtained at night disappears with a half-life of about 3 minutes at 37°. This decrease is prevented by acetyl coenzyme A, the cofactor in this reaction. It is possible that acetyl coenzyme A plays an important role in the physiological regulation of the rapid changes in the activity of pineal N-acetyltransferase that are regulated by environmental lighting.

2. Correlation of N-acetyltransferase with pineal melatonin: *In vivo* and *in vitro* studies have indicated that in rat and chicken pineal glands there is a very close correlation between the activity of N-acetyltransferase and the concentration of melatonin measured by bioassay. In contrast, there is no or a very poor correlation between melatonin and hydroxyindole-O-methyltransferase activity. Melatonin production, but not hydroxyindole-O-methyltransferase activity, can be increased many fold by N-acetylserotonin, the product of N-acetyltransferase. The available evidence also indicates that there is a very strict inverse relationship between the activity of N-acetyltransferase and the concentration of its precursor, serotonin. It is evident that N-acetyltransferase is the regulator of melatonin synthesis and the concentration of serotonin in the pineal gland.

3. Development of the diurnal rhythm in pineal N-acetyltransferase: Rat pineal N-acetyltransferase can be measured four days before birth. At four days after birth there is a detectable diurnal rhythm. At 21 days of age the amplitude of the rhythm has achieved adult proportions. At all ages examined, the activity of the enzyme is regulated by light acting via the eyes. The appearance of a rhythm in N-acetyltransferase follows closely the development of adenyl cyclase sensitivity to norepinephrine and suggests that this is an important factor in determining the appearance of a diurnal rhythm in N-acetyltransferase.

4. Phospholipid metabolism in the pineal gland: Cultured pineal glands incorporate ^{32}P into several phospholipids in postsynaptic membranes. Norepinephrine specifically increases by 2 fold the incorporation of label into one fraction which contains phosphatidyl serine and inositol. Alpha and beta blockers specifically decrease to 10% the incorporation of label into phosphatidyl choline and block the stimulation of phosphatidyl serine and inositol by norepinephrine. These studies suggest that phospholipids in membranes play an important role in the induced-receptor reaction.

Significance to Biomedical Research and the Program of the Institute: The findings of these investigations have provided a description of events whereby the release of the neurotransmitter, norepinephrine, acts to regulate the production of melatonin by the pineal gland and the levels of serotonin, another biogenic amine. The described mechanisms further our knowledge of how catecholamines regulate cellular metabolism through alterations in cyclic nucleotide metabolism. They may also be important in the control of serotonin metabolism in other areas of the brain.

Melatonin may be the antigonadotrophic substance which is responsible for gonadal atrophy observed following blinding and anosmia in the rat. A complete understanding of the mechanisms regulating the endogenous production of this compound may increase our understanding of the control of reproductive processes.

The mechanisms regulating diurnal biochemical rhythms which influence central nervous system activity and behavioral processes are poorly understood. Investigations on endogenous and norepinephrine-induced control of pineal gland serotonin metabolism may provide a valuable model for studying factors regulating central nervous system rhythms.

Proposed Course of Project: The study of the neural, endocrine, and developmental regulation of pineal N-acetyltransferase and hydroxyindole-O-methyltransferase will be continued. These studies will be expanded to include studies on human tissue. A method to measure melatonin in body fluids and tissue other than the pineal gland will be developed so that the physiological regulation of melatonin production, secretion, and excretion can be determined. Biochemical investigations of the molecular mechanisms regulating pineal N-acetyltransferase activity will focus on describing the mechanisms which regulate the rapid appearance and disappearance of N-acetyltransferase activity and on the mechanisms which determine the duration of induced periods of enzyme activity.

Honors and Awards: None

Publications:

1. Klein, D. C., Berg, G. R., and Weller, J.: Melatonin synthesis: Adenosine 3',5'-monophosphate and norepinephrine stimulate N-acetyltransferase. Science 168: 979-980, 1970.

2. Klein, D. C., and Berg, G. R.: Pineal gland: Stimulation of melatonin production by norepinephrine involves cyclic AMP-mediated stimulation of N-acetyltransferase. Advances in Biochemical Psychopharmacology 3: 241-263, 1970.

3. Klein, D. C., and Weller, J.: Input and output signals in a model neural system: The regulation of melatonin production in the pineal gland. In Vitro 6: 197-204, 1970.

4. Klein, D. C., and Weller, J.: Indole metabolism in the pineal gland: A circadian rhythm in N-acetyltransferase. Science 169: 1093-1095, 1970.

5. Berg, G. R., and Klein, D. C.: Non-cartesian pineal function. New Eng. J. Med. 284: 332, 1971.

6. Reiter, R. J., and Klein, D. C.: Observations on the pineal glands, the Harderian glands, the retinas, and the reproductive organs of adult female rats exposed to continuous light. J. Endocr., in press.

7. Berg, G. R., and Klein, D. C.: Pineal gland in organ culture II: The role of adenosine 3',5'-monophosphate in the regulation of radiolabelled melatonin production. Endocrinology, in press.

8. Klein, D. C., Reiter, R. J., and Weller, J. L.: Pineal N-acetyltransferase activity in blinded and anosmic male rats. Endocrinology, in press.

Serial No. HD-LB8(1)

1. Laboratory of Biomedical Sciences
2. Section on Developmental Pharmacology
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Developmental Pharmacology

Previous Serial Number: HD-LB8, and HD-LB17

Principal Investigator: D. W. Nebert

Other Investigators: J. E. Gielen
L. L. Bausserman
F. E. Goujon
W. F. Benedict

Cooperating Units: R. M. Friedman, Laboratory of Pathology, NCI
H. Green, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts
R. R. Bates, Biology Branch, NCI

Man Years

Total:	46/12
Professional:	36/12
Others:	10/12

Project Description:

Objectives: Membrane-bound mixed-function oxygenases are important in the metabolism of drugs, carcinogens, and insecticides, as well as many endogenous lipophilic substrates such as steroids, hemin, and bilirubin. We found that one such oxygenase, aryl hydrocarbon hydroxylase, is inducible in mammalian fetal liver cells grown in culture by polycyclic hydrocarbons and by phenobarbital. Whereas it is well known that polycyclic hydrocarbons and phenobarbital induce hepatic drug-metabolizing enzymes by different mechanisms, the precise effects evoked by these two classes of microsomal enzyme inducers are not understood. Cell culture thus provides an opportunity for studying the mechanisms of microsomal oxygenase induction in an experimental system which is more rigidly controlled than similar studies in the intact animal. Understanding the mechanisms by which drug-metabolizing enzymes are induced may elucidate important concepts in drug metabolism, developmental pharmacology, steroid biochemistry, chemical carcinogenesis, and entomology. The main objectives of this project are: (a) to compare the rates of hydroxylase synthesis and degradation and the physical properties of the phenobarbital-induced enzyme with those of the hydroxylase induced by polycyclic hydrocarbons, (b) to determine the extent to which hormones and nutrition affect the hydroxylase induction by either compound, (c) to characterize specific

changes in nuclear acidic proteins, cellular RNA, and microsomal membrane protein during the induction process by either compound, (d) to measure entry and binding of various inducers to specific receptor sites within the hepatocytes, (e) to develop methods for inhibiting or stimulating the sequence of events involved during the oxygenase induction by either class of inducers, and (f) to use this experimental system for studying the control of specific gene expression in higher organisms.

Methods Employed: The principal methods employed are: (1) recording spectrophotometry of turbid solutions, (2) enzyme assay involving spectrophotofluorometric determination of product formation, (3) radioisotopic measurements using tritium and carbon-14, (4) cell culture techniques, (5) differential centrifugation, (6) phase microscopy, (7) equilibrium dialysis, (8) polyacrylamide gel electrophoresis, (9) column chromatography, and (10) magnetic resonance techniques.

Major Findings: (1) The constitutive levels and the extent of aryl hydrocarbon hydroxylase induction in fetal tissues by benz[a]anthracene administration to the pregnant animal increase as a function of gestational age. These basal and inducible levels continue to increase after parturition, with the most marked rates of increase in the neonatal period. (2) In cultures derived from the whole mouse fetus, the maximally inducible level of hydroxylase activity in C57BL/6N cells is 4 to 6 times higher than that from DBA/2N cells. (3) There are no differences between these two cell types in the rate of uptake of benz[a]anthracene by the cells, in the total binding of polycyclic hydrocarbon to subcellular fractions, or in the rate of degradation of induced hydroxylase activity. (4) Compared with C57BL/6N cell cultures there is in the DBA/2N cells (a) a decreased extent of oxygenase induction, (b) diminished formation of total microsomal heme and CO-binding cytochromes, (c) less formation of a spectrally distinct CO-binding high-spin hemoprotein, and (d) indirect evidence for a decreased expression of induction-specific RNA. (5) The liver, lung, bowel, and kidney of C57BL/6N mice are highly inducible by polycyclic hydrocarbons, whereas those tissues from DBA/2N, NZB/BLN, and NZW/BLN strains are not; the response of hydroxylase induction by polycyclic hydrocarbons follows a Mendelian autosomal dominant pattern. (6) Aryl hydrocarbon hydroxylase activity is inducible in rat fetal hepatocytes in culture by phenobarbital, the insecticide 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane, benz[a]anthracene, or 3-methylcholanthrene. (7) Between the second and fourth day of age of the primary cultures, the maximal net rate at which the hydroxylase activity accumulates is about the same when phenobarbital, benz[a]anthracene, or 3-methylcholanthrene is in the growth medium at optimal concentrations. (8) The optimal inducing concentrations for each compound are 2.0 mM phenobarbital, 100 μM 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane, 13 μM benz[a]anthracene, and 0.75 μM 3-methylcholanthrene. (9) The kinetics of the oxygenase induction and degradation are the same in liver cell cultures exposed to either phenobarbital or benz[a]anthracene; the time required for doubling the hydroxylase activity is about 3.0 hours, and the initial half-life of the induced enzyme system is about 10.5 hours. (10) An additive effect is obtained when either phenobarbital or 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane is present with a polycyclic hydrocarbon in the medium but not when the hepatocytes are treated with phenobarbital plus 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane or with the combination of

benz[a]anthracene and 3-methylcholanthrene. (11) The apparent Michaelis constants for the control hydroxylase and for the enzyme system inducible by either phenobarbital or benz[a]anthracene range between 2 and 4 μM in the presence of the substrate benzo[a]pyrene. (12) Compared with the induction process by benz[a]anthracene, aryl hydrocarbon hydroxylase induction in fetal rat hepatocyte cultures by phenobarbital is more sensitive to low doses of either actinomycin D or cycloheximide; by extrapolation to other studies in vivo and in cell culture, nucleolar synthesis of ribosomal RNA may be of more importance in the process of microsomal enzyme induction by phenobarbital than that by benz[a]anthracene. (13) Degradation of the phenobarbital- or benz[a]anthracene-induced hydroxylase activity is the same whether the enzyme system is maximally or half-maximally induced: the half-life is 10.5 ± 3.6 hours in fresh control medium and 8.5 ± 2.5 hours in the presence of high levels of cycloheximide. (14) Actinomycin D stabilizes or slightly stimulates the enzyme activity in fetal liver cells previously treated with either phenobarbital or benz[a]anthracene. (15) The continued presence of either inducer in hepatocytes exposed to high levels of cycloheximide or actinomycin D also stabilizes the phenobarbital- or benz[a]anthracene-induced hydroxylase activity. (16) Actinomycin D does not prevent the induction of oxidase activity in cells previously treated with cycloheximide and either inducer. (17) Therefore, the primary action of phenobarbital, like that of benz[a]-anthracene, is at the level of transcription; with either inducer there is also a secondary effect of lesser importance at the posttranslational level in which the normal rate of decay of the induced enzyme is delayed. (18) In fetal mouse cell cultures treated with highly purified interferon, a relationship exists between the high antiviral activity and a stimulation in the rate of hydroxylase induction by benz[a]anthracene. (19) The HLE hybrid cell line, established from the parental mouse 3T3-4(E) and human D98/AH2 lines, has inducible hydroxylase activity similar to that found in the mouse parental line; the human parental line is noninducible by benz[a]anthracene. (20) Hydroxylase activity is present in mouse skin and inducible by administration of polycyclic hydrocarbons; the control and inducible enzyme activities are slightly lower in castrated male mice which have received subcutaneous implants of 17-β-estradiol. (21) In mouse fetal cell cultures, 17-β-estradiol in the growth medium prevents hydroxylase induction only at concentrations 6 to 60 times greater than the level of polycyclic hydrocarbon inducer in the medium. (22) In a cell-free in vitro system, both estradiol and testosterone competitively inhibit polycyclic hydrocarbon hydroxylation, even at levels of steroid that are lower than that of the polycyclic hydrocarbon inducer. (23) Benz[a]anthracene and α-naphthoflavone are competitive inhibitors of metabolism of the carcinogen 7,12-dimethylbenz[a]anthracene. (24) The metabolite 3-hydroxybenzo[a]pyrene, as is the case with the parent compound benzo[a]pyrene, transforms fetal hamster fibroblasts. (25) In hamster cell cultures aneuploidy is associated with transformation, whereas chromatid breaks are correlated with polycyclic hydrocarbon-produced toxicity.

Significance to Biomedical Research and the Program of the Institute: Phenobarbital is the most commonly used drug from a class of more than 200 drugs, chemicals, and insecticides that induce microsomal enzymes apparently by the same mechanism. Administration of any of these substances to the pregnant animal causes transplacental induction of these mixed-function oxygenases in the fetal liver. These enzyme systems metabolize steroids, hemin, cholesterol,

fatty acids, indoles, sympathomimetic amines, thyroxine, and bilirubin, as well as lipophilic foreign substrates. Administration of polycyclic hydrocarbons to the pregnant animal produces induction of this enzyme activity in the placenta and also transplacental stimulation of the hydroxylase in several fetal tissues. Cigarette smoking is associated with increases in human placental aryl hydrocarbon hydroxylase activity. Consequently, studies involving the development and extent of induction of mixed-function oxygenases in fetal tissues and in the placenta during various periods of gestation, are relevant to the program of this Institute in the following areas: (1) possible effects of environmental pollutants (i.e., polycyclic hydrocarbons and insecticides) and various drugs on normal fetal growth and development and on the health of the pregnant woman, (2) mechanisms of "pharmacological immaturity" of the premature and newborn, (3) mechanisms of disease of the fetus and newborn concerning oxygenative and conjugative metabolism of normal body substrates such as steroids and bilirubin, and (4) possible therapeutic and diagnostic procedures for aiding and predicting pharmacogenetic defects in the fetus and neonate.

Proposed Course of Project: (1) Further delineation of differences between mixed-function oxygenase induction by phenobarbital and that by polycyclic hydrocarbons, (2) characterization of specific and nonspecific binding of these various inducers to subcellular sites, (3) examination of the effects of hormones and nutrition on hydroxylase induction by either type of inducers, (4) study of various inhibitors on the induction process in cell culture, (5) characterization of the various components of the membrane-bound "hydroxylase" system, (6) analysis of differences in product formation and biophysical properties between the control, phenobarbital, and polycyclic hydrocarbon-induced oxygenases, and (7) extrapolation of these studies to formulate a general theory for explaining the mechanisms by which gene expression is controlled in mammalian cells.

Honors and Awards: None

Publications:

1. Nebert, D. W., and Bausserman, L. L.: Fate of inducer during induction of aryl hydrocarbon hydroxylase activity in mammalian cell culture. I. Intracellular entry, binding, distribution and metabolism. Molec. Pharmacol. 6: 293-303, 1970.

2. Nebert, D. W., and Bausserman, L. L.: Fate of inducer during induction of aryl hydrocarbon hydroxylase activity in mammalian cell culture. II. Intracellular content of polycyclic hydrocarbon during enzyme induction and decay. Molec. Pharmacol. 6: 304-314, 1970.

3. Nebert, D. W., and Bausserman, L. L.: Genetic differences in the extent of aryl hydrocarbon hydroxylase induction in mouse fetal cell cultures. J. Biol. Chem. 245: 6373-6382, 1970.

4. Nebert, D. W., Bausserman, L. L., and Bates, R. R.: Effect of estradiol and testosterone on aryl hydrocarbon hydroxylase activity in mouse tissues in vivo and in cell culture. Int. J. Cancer 6: 470-480, 1970.

5. Gielen, J. E., and Nebert, D. W.: Microsomal hydroxylase induction in liver cell culture by phenobarbital, polycyclic hydrocarbons, and p,p'-DDT. Science 172: 167-169, 1971.

6. Nebert, D. W., and Bausserman, L. L.: Aryl hydrocarbon hydroxylase induction in cell cultures as a function of gene expression. Ann. N. Y. Acad. Sci., in press.

7. Friedman, R. M., and Nebert, D. W.: Viral interference: The mechanism of action of interferon. In: Regulation of Cell Metabolism: Organizational and Pharmacological Aspects on the Molecular Level. New York, Academic Press, 1971, in press.

8. Gielen, J. E., and Nebert, D. W.: Aryl hydrocarbon hydroxylase induction in mammalian liver cell culture. I. Stimulation of enzyme activity in nonhepatic cells and in hepatic cells by phenobarbital polycyclic hydrocarbons, and 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane. J. Biol. Chem., in press.

9. Nebert, D. W., and Gielen, J. E.: Aryl hydrocarbon hydroxylase induction in mammalian liver cell culture. II. Effects of actinomycin D and cycloheximide on induction processes by phenobarbital or polycyclic hydrocarbons. J. Biol. Chem., in press.

10. Nebert, D. W., and Gielen, J. E.: Genetic regulation of aryl hydrocarbon hydroxylase induction. Fed. Proc., in press.

Serial No. HD-LB3

1. Laboratory of Biomedical Sciences
2. Section on Molecular Structure
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Analytical Techniques and Instrumentation
a. Amino Acid Analysis
b. Automatic Monitoring of Chromatographic Experiments

Previous Serial Number: Same

Principal Investigator: E. Gross

Other Investigators: H. H. Kiltz

Cooperating Units: Systems Maintenance Section, DRS-BEI, NIH
Division of Computer Research and Technology, NIH

Man Years

Total:	6/12
Professional:	6/12
Others:	0

Project Description:

Objectives: (1) Analysis of the amino acid composition of peptides and proteins; (2) improvement of existing analytical instruments and automation of techniques which require many time-consuming operations; (3) adaptation of conventional amino acid analyzers to the separation of unusual amino acids; (4) operation of multiple sample applicators, to be followed by an automatic sample applicator of sufficient capacity that the amino acid analyzer will be operated continuously for prolonged periods of time without attendance.

Methods Employed: Ionexchange chromatography using custom resins of spherical shape, colorimetry, and electronic data processing.

Major Findings: The one-column system for ionexchange chromatography of amino acids--originally a prerequisite for the rational implementation of automatic sample application--is most useful in the separation of unusual amino acid mixtures. These are but a few examples: (1) hydrolyzates of subtilin : separation of lanthionine and β-methyllanthionine from glutamic acid; (2) mercaptan addition products of subtilin:separation of S-benzyl-cysteine from isomers of β-methyl-S-benzylcysteine; (3) identification and subsequent isolation of lysino-alanine and β-hydroxyaspartic acid from cinnamycin. The operation of the automatic sample applicator guarantees optimal utilization of the amino acid analyzer; a second applicator has been constructed.

Significance to Biomedical Research and the Program of the Institute: Large numbers of amino acid analyses are required in the course of the structural elucidation of peptides and proteins. The extensive use of the amino acid analyzer has significantly contributed to the structural elucidation of nisin and subtilin, peptides with unusual structural features. Adaptations of the amino acid analyzer to the detection and quantitation of rare amino acids found in nisin and subtilin continue to facilitate the characterization of the two peptides. Unique degradation products are quickly determined by related approaches. Nisin is membrane-active and capable of inducing fetal resorption; so are structural modifications of the molecule. Precise knowledge of these modifications is essential for the interpretation of structure-function relationships and only possible with the help of large numbers of amino acid analyses.

Proposed Course of Project: (1) Mechanical features of the Automatic Sample Applicator will be refined. A second automatic sample applicator will be added for the alternating operation of one pair of columns and further reductions in the time requirement for individual analyses. The amino acid analyzer equipped with the Automatic Sample Applicator will be linked to a small computer capable of feedback and process control in order to demonstrate advanced automatic techniques of laboratory automation and their application to protein chemistry. (2) The hydrolysis of peptides and proteins will be automated and samples directly advanced to the Automatic Sample Applicator for column loading. (3) Other laboratory processes will be further automated in collaboration with the Division of Computer Research and Technology. (4) The sequence analysis of peptides and proteins will be automated by (a) utilizing solid supports, (b) employing mass spectroscopic techniques.

Honors and Awards: None

Publications: None

Serial No. HD-LB14

1. Laboratory of Biomedical Sciences
2. Section on Molecular Structure
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cell Organelles: Formation, Isolation, Characterization, and Function Under Normal and Abnormal Conditions

Previous Serial Number: Same

Principal Investigator: E. Gross

Other Investigators: H. Rehner
K. Collins

Cooperating Units: Behzad Mohit, Clinical Center, Clinical Pathology Department, NIH

Man Years

Total:	25/12
Professional:	25/12
Others:	0

Project Description:

Objectives: (1) Isolation and characterization of lysosomes; (2) study of the membrane of rat liver lysosomes: chemical composition, reactivity, permeability, structural protein(s), membrane-bound enzymes; (3) constituents of rat liver lysosomes: isolation and characterization of enzymes, basic proteins, and other components; (4) correlation of lysosomal enzymes and their function with the life cycle of the cell under normal and pathological conditions; (5) analysis of lysosomal enzyme levels in the human placenta at various stages of gestation.

Methods Employed: Ultracentrifugation, zonal centrifugation, carrier-free electrophoresis, techniques of enzyme analysis, electron microscopy, amino acid analysis.

Major Findings: The isolation of detergent-free rat liver lysosomes has been further improved by the introduction of the cell disruption bomb in the homogenization step. The structural protein of the lysosomal membrane has been purified by column chromatography and countercurrent distribution. Amino acid analytical and preliminary physical data indicate that the protein is of a molecular weight of approximately 6000. The membrane effect of nisin on cultured mouse erythrocytes is concentration dependent. It diminishes above nisin levels of 4×10^{-4} mmoles/ml possibly due to aggregation and concomitant autoinhibition by reducing the number of available α,β-unsaturations.

Significance to Biomedical Research and the Program of the Institute: Lysosomes occupy a unique position in the life cycle of the cell. The large number of hydrolases present in the organelle perform a variety of important functions under normal and abnormal cellular conditions. Profound physiological events, such as those of reproduction, may depend upon the availability of lysosomal enzymes. Extensive investigations of lysosomes in cells from different organs of the maternal as well as the fetal organism will contribute to the better understanding of the role of this organelle during development.

Proposed Course of Project: (1) Improvement and application of techniques available for the isolation of lysosomes from different types of developing tissues; (2) isolation and identification of lysosomal enzymes and other protein constituents; (3) elucidation of the structure and function of the lysosomal membrane; (4) study of lysosomes under normal and abnormal conditions; (5) investigation of the effect of low molecular weight peptides and proteins on the lysosomal membrane; and (6) correlation of the structure of membrane-active compounds with structural features of the lysosomal membrane.

Honors and Awards: None

Publications: None

Serial No. HD-LB15

1. Laboratory of Biomedical Sciences
2. Section on Molecular Structure
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Fetal Resorption

Previous Serial Number: Same

Principal Investigator: E. Gross

Other Investigators: None

Cooperating Units: K. C. Snell, Laboratory of Pathology, NCI

Man Years

Total:	3/12
Professional:	3/12
Others:	0

Project Description:

Objectives: (1) Isolation, purification, and structural elucidation of substances capable of inducing fetal resorption; (2) correlation of the structure and mode of action of these compounds; (3) identification of the cellular constituents affected by and/or contributing to fetal resorption.

Methods Employed: (1) Microbiological culturing techniques to provide compounds capable of inducing fetal resorption; (2) column chromatography and countercurrent distribution in purification steps; (3) enzymatic and nonenzymatic methods in combination with amino acid analysis to determine the structure of peptides which induce fetal resorption; (4) animal experiments to test the activity of substances for which the induction of fetal resorption has been predicted.

Major Findings: Structural considerations led to the conclusion that nisin is capable of inducing fetal resorption. In experiments with rats, intraperitoneal administration of nisin induced fetal resorption to the extent of 90% as late as day 15 of the pregnancy. Nisin is also effective in early stages of gestation. It prevents the implantation of the fertilized egg when given prior to day 5 of the rat pregnancy. Nisin induces resorption in the rabbit.

Significance to Biomedical Research and the Program of the Institute: The regulatory mechanisms operative at many stages of intrauterine development are not fully understood. Studies with substances with novel types of

structures will serve to (1) identify physiological processes that contribute to the normal course of pregnancy, (2) detect undesirable metabolites which affect gestation adversely, and (3) offer chemotherapeutic approaches to pregnancy regulation.

Proposed Course of Project: (1) Structural elucidation of peptides capable of inducing fetal resorption; (2) correlation of structure and function during this physiological event; (3) optimizing conditions for fetal resorption by establishing appropriate dose levels, routes of administration, and timing of administration; (4) histological studies; (5) investigation of the effects of previously induced resorptions on subsequent pregnancies; (6) extension of these studies to primates.

Honors and Awards: None

Publications: None

Serial No. HD-LB19

1. Laboratory of Biomedical Sciences
2. Section on Molecular Structure
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chemical Modification of Amino Acids. Selective Nonenzymatic Cleavage of Peptide Bonds. Structure-Function Relationship of Peptide-Antibiotics and Enzymes. Peptide Synthesis.

Previous Serial Number: HD-LBM16

Principal Investigator: E. Gross

Other Investigators:
J. L. Morell
A. Twarmoh
H. H. Kiltz
M. Morishita
J. Brown

Cooperating Units:
L. C. Craig, Rockefeller University, New York, New York
F. H. White, Jr., Laboratory of Biochemistry, NHLI

Man Years

Total:	41/12
Professional:	41/12
Others:	0

Project Description:

Objectives: (1) Chemical modification of reactive groups in polyfunctional amino acids; (2) selective cleavage of peptide bonds (nonenzymatic fragmentation) by application of chemical reactions; (3) production (by culturing microorganisms), isolation, and purification of peptide antibiotics and of enzymes; (4) fragmentation of antibiotics and enzymes with the objective of studying residual activity; (5) synthesis of enzyme inhibitors and study of inhibitory effects, identification of the site of inhibition and of loci responsible for enzyme activity; (6) structural elucidation of peptide antibiotics and correlation of structure and function; (7) peptide synthesis.

Methods Employed: (1) Oxidants for the modification of tryptophan and the cleavage of the tryptophyl peptide bond; (2) cyanogen bromide for (a) the cleavage of methionyl peptide bonds, (b) the cleavage of the N-aminoacyl bond of S-alkylcysteine, (c) the removal of the water-soluble amino protecting group acetylmethionine in peptide synthesis and modifications of other thioether amino acids; (3) reduction of β-esters of aspartic acid in peptides and proteins and cleavage of the resulting homoseryl peptide bond; (4) various

techniques for the synthesis of peptides including the automation of the solid phase method; (5) advanced separation and analytical techniques: carrier-free electrophoresis, countercurrent distribution, automatic sample application in amino acid analysis; (6) use of electronic equipment in data handling and process control.

Major Findings: (1) Cyanogen Bromide Reaction: Starting material has been prepared for the synthesis of valyldehydroalanyllysine and for a conclusive study of the causes for the inhibition of the reaction of cyanogen bromide with the sequence valyl-S-methylcysteinyllysine in modified nisin. (2) Nisin: The structure of nisin has been established with the exception of the assignment of two sulfide bridges and the clarification of a rearrangement reaction. The H_2N-terminal fragment was cleaved with trypsin and the following structures established for the fragments.

H_2N-ILEU-ABA-ALA-ILEU-ALA-LEU-DHA-DHAB-GLY-PRO-ALA-LYS-COOH

H_2N-ABA-GLY-ALA-LEU-MET-GLY-ALA-AN-MET-COOH

The structure of nisin is shown in Figure 1. (3) Subtilin: Cleavage of the peptide with trypsin and thermolysine results in the formation of fragments suitable for the structural determination. The partial structures of subtilin reads as follows: H_2N-TRY-LY-LAN, β-CH_3-LAN_2, GLU_2, PRO, GLY_2, VAL, LEU_2, ALA-PHE, ASP, β-CH_3-LAN_2-GLU, LEU_2-LYS-ILEU-DHA-LYS-COOH. (4) Peptide Synthesis: Gramicidin B and C have been synthesized by the solid phase method; the COOH-terminal fragment of nisin has been converted to the tris-prolyl derivative to study the effect of high-energy radiation on the α,β-unsaturated amino acid; various derivatives of nisin and of fragments of nisin have been prepared for the study of induced fetal resorption. (5) The purification of nisin and of fragments of nisin has been simplified by the elimination of the chromatography on Sephadex columns.

Significance to Biomedical Research and the Program of the Institute: (1) The Cyanogen Bromide Reaction--highly selectively and widely applied to the structural elucidation of peptides and proteins--has been applied extensively to provide water-soluble fragments of nisin for fetal resorption studies. (2) Details of the structure of nisin are important to know in view of the biological activities displayed by the peptide. The implied working hypothesis of the interaction of α,β-unsaturated amino acids and vital sulfhydryl groups is possibly of broad biological significance. In turn, the reaction is reversible and linked to the formation of ketoacids and amides, frequently encountered constituents of many biological systems. These structural considerations form the basis of the studies of the induced fetal resorption by nisin. (3) The synthesis of peptides with ion-complexing ability, such as the gramicidins A, B, and C has long range implications for the study of compounds which affect fetal development.

Proposed Course of Project: (1) Continued application of the Cyanogen Bromide Reaction; (2) chemical modification of amino acids and cleavage of peptide bonds: (a) tryptophan: application of selective oxidants to the cleavage of tryptophyl peptide bonds, (b) methionine: study of the selective suppression of the cleavage of methionyl peptide bonds upon treatment with

cyanogen bromide, (c) S-alkylcysteine: fragmentation of reduced and S-alkylated peptides and proteins; fragmentation of peptides and proteins subsequent to mercaptan addition to α,β-unsaturated amino acids, (d) aspartic acid: to be developed to a generally applicable method of nonenzymatic fragmentation; (3) nisin: continuation of the structural elucidation:assignment of the remaining sulfide bonds and determination of optical configurations of partial sequences and study of physiological properties; (4) subtilin: elucidation of the primary structure; study of phylogenetic aspects of the structure of antibiotics (comparison of nisin and subtilin); (5) peptide synthesis: synthesis of model peptides for the development of nonenzymatic fragmentation techniques; synthesis of peptides of biological interest via the solid phase method.

Honors and Awards: None

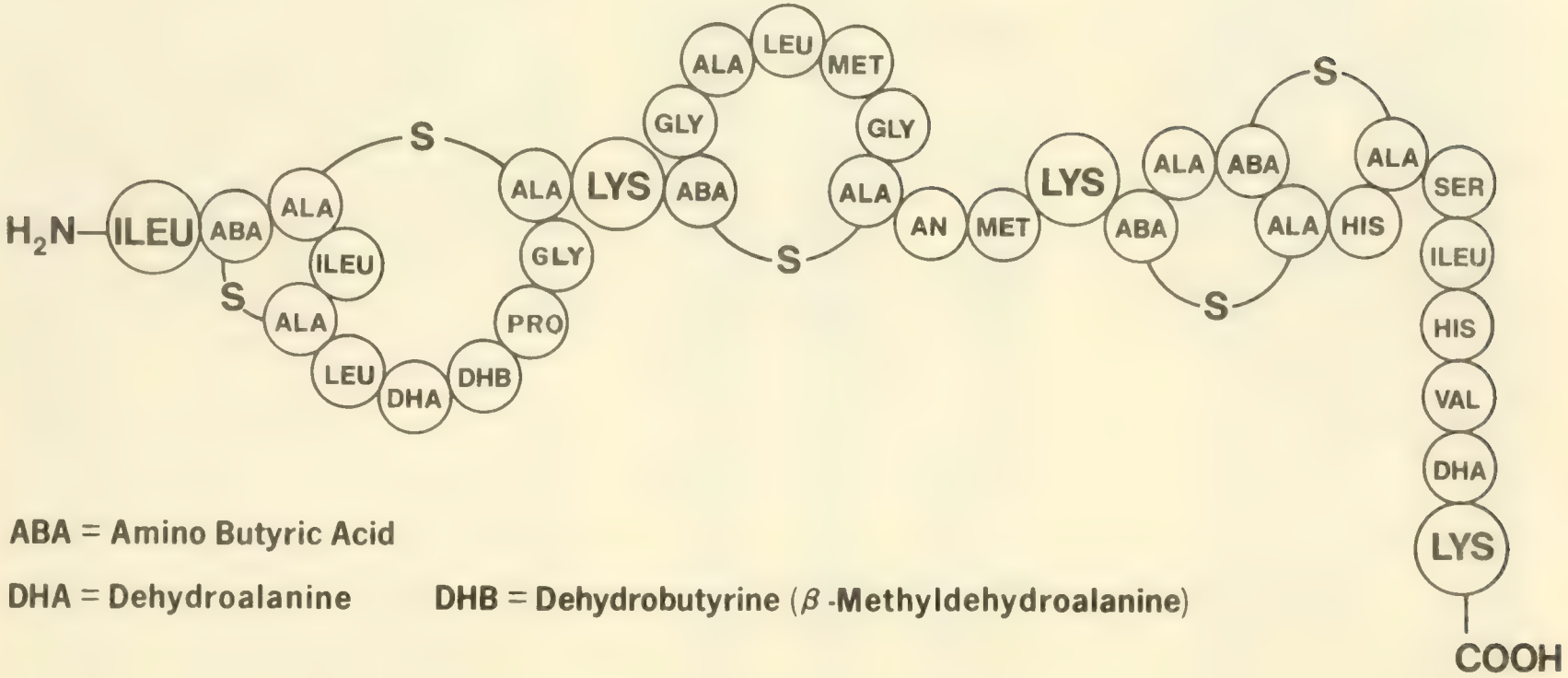
Publications:

1. Gross, E., and Morell, J. L.: Structural studies on nisin. Proceedings First American Peptide Symposium. August, 1968, Yale University, New Haven, Connecticut. New York, New York, Marcel Dekker, Inc., 1970, pp. 389-410.

2. Gross, E., and Morell, J. L.: Peptides with α,β-unsaturated amino acid. Proceedings Tenth European Peptide Symposium, Abano Terme, Italy, September 7-13, 1969. E. Scoffone (Ed.), in press.

3. Gross, E., and Morell, J. L.: Nisin: The assignment of sulfide bridges of β-methyllanthionine to a novel bicyclic structure of identical ring size. J. Amer. Chem. Soc. 92: 2919-2920, 1970.

FIG. I. THE STRUCTURE OF NISIN



ABA = Amino Butyric Acid

DHA = Dehydroalanine DHB = Dehydrobutyrine (β-Methyldehydroalanine)

Serial No. HD-LB7(c)
1. Laboratory of Biomedical Sciences
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Viral Diseases of the Nervous System

Previous Serial Number: Same

Principal Investigator: L. W. Catalano, Jr.

Other Investigators: J. L. Sever
S. Baron

Cooperating Units: J. L. Sever, Section on Infectious Diseases, Perinatal Research Branch, NINDS
S. Baron, Laboratory of Viral Diseases, NIAID
J. A. Bellanti, Georgetown University School of Medicine, Washington, D. C.
L. Johnson, Harvard Medical School, Boston, Massachusetts

Man Years

Total:	2/12
Professional:	1/12
Others:	1/12

Project Description: Description of this project is the same as in the Progress Report July 1, 1969 through June 30, 1970. This project was terminated June 30, 1970. The Man Years above represents time spent in revision and typing of manuscripts.

Publications:

1. Catalano, L. W., Jr., Moossy, J., and Sell, S.: Viral interference in the brains of mice infected subcutaneously with herpes simplex virus. Proc. Soc. Exp. Biol. Med. 135: 509-514, 1970.

2. Bellanti, J. A., Catalano, L. W., Jr., and Chambers, R. W.: Herpes simplex encephalitis: Virologic and serologic study of a patient treated with an interferon inducer. J. Pediat. 78: 136-145, 1971.

3. Nahmias, A. J., London, W. T., Catalano, L. W., Fuccillo, D. A., Sever, J. L., and C. Graham: Genital herpesvirus hominis type 2 infection: An experimental model in cebus monkeys. Science 171: 297-298, 1971.

4. London, W. T., Catalano, L. W., Jr., Nahmias, A. J., Fuccillo, D. A., and Sever, J. L.: Genital Herpesvirus hominis type 2 infection of monkeys. Obstet. Gynec. 37: 501-509, 1971.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971
Children's Diagnostic & Study Branch

During the past fiscal year the staff of this branch has been seriously depleted. During most of the year it has amounted to 3 professionals, assisted by one full-time and one part-time technical support person. The program content has been related in large measure to cytogenetic studies, studies of lineage through dermatoglyphics and various proteins of plasma, and reading disability.

Attempts have been made to further delineate the frequency of the XYY karyotype among 500 male offenders sent to the juvenile court. It is of interest that despite the published evidence that there is an increased frequency of this karyotype among juvenile offenders, none was detected.

Culture of strains of fibroblasts obtained from skin biopsy specimens from a number of subjects with karyotypic abnormalities have been established. These lines are being maintained for gene mapping studies.

A study of nine institutionalized negro patients with Down's Syndrome, six out of seven with trysomey 21, indicated that they carried the Australia antigen; however, the two patients with G/C translocation did not. Only two of fourteen patients in the same institution with normal karyotypes carried the antigen. This indicates a far higher incidence of the Australian antigen among 21 trysomey patients than in other forms of Down's Syndrome or in normal controls. It may have significance with regard to chronic viral carrier states or even become another type of marker for trysomey 21. The chromosome structure and karyotype of two patients with cystinosis have been studied. They, their parents and siblings were found to be normal.

Dermatoglyphics have been studied in normal caucasian Americans, normal black Americans, Papuan natives of New Guinea, and of West New Guinea and of Cypriots, Micronesians, the Kukukuku Fore peoples of New Guinea, Tongariki Islanders in New Hebrides.

Study of perceptual functioning in a group of children identified as having reading disability were screened to eliminate those with discernable neurological problems. After screening it was found that only one of the 54 was a female. All subjects showed superior ability to perform visual ordering of material and information. The studies suggest that there is a hereditary component transmitted by an x-link recessive gene which confers upon males this ordering superiority but in addition confers inefficiency in reading. This is presumably a result of inefficient facility for three dimensional coding and processing. This compromises their ability to see the letters b-p-q-d. It is possible that English orthography is particularly difficult for these males, for in Japan where different script is used, the incidence of reading disability is less than 1% whereas in American school children (mainly among males) it may be as high as 5%.

Studies in a patient with the Prader-Willi Syndrome in which there is anatomic

evidence of loss of hypothalamic tissue it was possible to demonstrate a change in the circadian periodicities of a variety of biologic mordalities. The studies in this subject subsequently extended through collaboration to include two other similarly affected patients, strongly indicate that the hypothalamic region controls circadian rhythm in human subjects.

Serial No. HD-CD1(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Relation Between Minor Physical Anomalies and Genetic Factors

Previous Serial Number: Same

Principal Investigators: Matti S. Al-Aish, Ph.D., Research Cytogeneticist
Chris C. Plato, M.S., Geneticist

Other Investigators: C. F. Halverson, Jr., Ph.D.
Mary F. Waldrop, M.A.
P. V. Cardon, M.D.
H. Gold, M.D.
National Institute of Mental Health

Cooperating Unit: Child Research Branch, National Institute of Mental Health, Bethesda, Maryland

Man Years

Total	: 0.05
Professional	: 0.05
Other	: 0.00

Project Description:

Objectives: This study is a search for genetic factors (cytogenetic, blood groups and dermatoglyphics) associated with existence of minor physical anomalies (such as hypertelorism, epicanthus, high-arched palate and curved fifth finger) and hyperactive behavior in young children.

Methods Employed: Sixty boys and girls age 7½-8½ years whose behavior was studied originally in the newborn period (1960-1962) and again during 1962-1964 were chosen for this study. These sixty children are divided into four subgroups as follows: (A) High activity associated with three or more minor physical anomalies. (B) High activity associated with one physical anomaly. (C) Low activity associated with three or more physical anomalies. (D) Low activity associated with one physical anomaly. Chromosomal analysis of each subject was performed on metaphases from cultured peripheral blood. For blood grouping each sample was typed by commercial antisera for ABO, Rh, Kell, Duffy, and other

rare systems. The dermatoglyphics were taken by the Faurot "inkless method." Both finger and palm patterns were evaluated.

Major Findings: A chromosome marker has been found in association with neuroblastoma in one of the subjects. A manuscript for this finding is being prepared. An abstract of this finding has been published in the proceedings of the Thirteenth Annual Meeting of the American Society of Hematology.

Significance to Biomedical Research and the Program of the Institute: This pilot project is designed to search for genetic associations with hyperactive behavior and/or minor physical anomalies.

Proposed Course of Project: This pilot project has been completed. Data analysis and manuscript preparation are underway.

Honors and Awards: None

Publications: None.

Serial No. HD-CD4(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cytogenetic Correlates of Antisocial Behavior

Previous Serial Number: Same

Principal Investigators: W. Edwin Dodson, M.D., Children's Hospital, Boston, Massachusetts
Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Other Investigators: Duane Alexander, M.D., Kennedy Institute, Baltimore, Maryland
Jean Symmes, Ph.D., Research Psychologist
Judith Rapoport, M.D., Department of Psychiatry, Georgetown University Medical School

Cooperating Units: The Circuit Court of Prince George's County, Division of Juvenile Causes, Mr. David Gray Ross, Master of Juvenile Causes
The Juvenile Court of the District of Columbia, The Honorable Morris Miller, Chief Judge

Man Years

Total	:	0.25
Professional	:	0.20
Other	:	0.05

Project Description:

Objectives: This study surveyed young male offenders to assess the incidence of the XYY chromosome anomaly, and identify such individuals for psychologic and psychiatric characterization, and for possible endocrinologic, genetic and therapeutic investigations.

Methods Employed: Blood samples were obtained from 500 males, age 8-16, facing charges in Juvenile Court. Lymphocytes were cultured and karyotyped using standard cytogenetic techniques.

Major Findings: No XYY patients were identified. One abnormal karyotype was found, a boy with a minute extra chromosome and no physical or mental abnormalities. One XYY patient was studied on referral and found to have

marked behavioral aberrations.

Significance to Biomedical Research and the Program of the Institute: Among selected antisocial males there is a clustering of aneuploidy of the Y chromosome. Men with this abnormality differ from their fellow inmates in that they are significantly taller, begin their antisocial behavior earlier, and their siblings have a lower crime rate. Recent reports suggest that these men have elevated L.H. and testosterone excretion. The identification of a population of affected boys for further study and therapeutic trials would have importance from genetic, developmental, behavioral, and social perspectives.

Proposed Course of Project: The study is complete and a manuscript has been mailed to Pediatric Research for publication.

Honors and Awards: None

Publications: None

Serial No. HD-CD7(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The DiGeorge Syndrome: Evaluation of a Patient with Thymic Aplasia

Previous Serial Number: Same

Principal Investigators: W. Edwin Dodson, M.D., Children's Hospital, Boston, Massachusetts
Duane Alexander, M.D., Kennedy Institute, Baltimore, Maryland

Other Investigators: Joseph Kennedy, M.D.
Melvin Museles, M.D.
National Naval Medical Center
Matti S. Al-Aish, Ph.D., Research Cytogeneticist
Felix de la Cruz, M.D.

Cooperating Units: National Naval Medical Center, Department of Pediatrics, Bethesda, Maryland

Man Years

Total	: 0.00
Professional	: 0.00
Other	: 0.00

Project Description: Complete.

Honors and Awards: None

Publications: Dodson, W. E., Alexander, D. F., Al-Aish, M. S., and de la Cruz, F.: The DiGeorge Syndrome. Lancet i: 574-575, 1969.

Serial No. HD-CD8(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics and the Determination of Twin Zygosity

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
J. Theodore Schwartz, M.D., National Eye Institute
J. D. Niswander, D.D.S., National Institute of Dental Research

Other Investigators: None

Cooperating Unit: Field and Developmental Research, National Eye Institute

Man Years

Total	:	0.10
Professional	:	0.05
Other	:	0.05

Project Description:

Objectives: This study will attempt to establish criteria for the determination of zygosity in twins from the dermatoglyphic data.

Methods Employed: Finger and palm prints are taken from each pair of twins of known zygosity from blood typing. The study will involve over 500 pairs of twins participating in the ophthalmologic investigations conducted by Dr. J. T. Schwartz (NEI(CF)-70E001). The following dermatoglyphic aspects will be studied in detail: fingerprint patterns, main line terminations, presence of interdigital patterns, amplitude of the atd angle, presence of accessory triradii and presence of simian crease.

Major Findings: Project is still in progress.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the extent of congruency of dermatoglyphics in monozygotic and dizygotic twins may permit reduction of the probability of error involved in the diagnosis of zygosity using blood typing alone.

Proposed Course of Project: The collected prints are presently being evaluated.

Honors and Awards: none.

Publications: none.

Serial No. HD-CD9(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics of the Islanders of Tongariki in New Hebrides

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigator: D. Carleton Gajdusek, M.D., Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Cooperating Unit: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.05
Professional	:	0.05
Other	:	0.00

Project Description: Complete

Honors and Awards: None

Publications: Plato, C.C., and Gajdusek, C. D.: Dermatoglyphics of the Natives of Tongariki in the New Hebrides. Hum. Biol. in Ocean., May, 1971.

Serial No. HD-MR9(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cytogenetic Evaluation of Children with Congenital Anomalies and Mental Retardation

Previous Serial Number: Same

Principal Investigator: Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Other Investigators: None

Cooperating Units: Departments of Pediatrics and Endocrinology, National Naval Medical Center, U.S. Naval Hospital, Bethesda, Maryland
DeWitt Army Hospital, Andrew Rader Army Clinic, Fort Myer, Andrews Air Force Base
Hospital for Sick Children, Washington, D. C.

Man Years

Total	: 0.35
Professional	: 0.25
Other	: 0.10

Project Description:

Objectives: This project is an ongoing study to identify and delineate cytogenetic abnormalities and their clinical and behavioral correlates, and to establish in tissue culture fibroblast lines from patients with rare chromosome constitutions.

Methods Employed: Buccal smears and karyotypes from bone marrow, peripheral blood, and skin fibroblasts are studied for chromosome anomalies.

Major Findings: Cytogenetic abnormalities have been detected in patients with a variety of clinical disorders. Study of the patient with Apert's Syndrome and a chromosome translocation has been completed and a manuscript submitted for publication. The patient with Monosomy G discovered here in 1966 was restudied and no evidence of mosaicism or translocation was found; tissue culture strains of fibroblasts from skin biopsy have been established for future studies on gene mapping. Two new patients with testicular feminization have been detected. A patient with a ring D chromosome has

been detected and haptoglobin typing is pending. A family with a translocation of the short arms of chromosome 21 to the short arms of a D group chromosome (21p-; Dp+) with balanced carriers, partial trisomy (21p+) and partial monosomy (21p-) have been studied, and skin is being obtained for future gene mapping studies. Manuscripts are being completed for patients detected earlier with the anomalies 48,XYY,G+ (XYY Mongol) and 46,XO,?Xi (Turner's syndrome variant).

Significance to Biomedical Research and the Program of the Institute: This source of material provides patients of cytogenetic interest as well as training opportunities in recognition and characterization of patients with cytogenetic abnormalities. Establishing tissue culture lines of fibroblasts from patients with rare cytogenetic constitutions is fundamental to a program of chromosome mapping.

Proposed Course of Project: The screening of mentally retarded children for chromosome and dermatoglyphic abnormalities will continue. Further appropriate studies of patients, parents, siblings, and other relatives will follow detection of significant abnormalities.

Honors and Awards: None

Publications: Dodson, W. E., Museles, M., Kennedy, J. L., and Al-Aish, M.S.: Acrocephalosyndactylia associated with chromosomal translocation, 46,XX,t (2p-;Cq+). Amer. J. of Dis. of Child. 120: 360-362, 1970.

Bachman, R., de la Cruz, F., Al-Aish, M. S., and Santell, F.: Short arm deletion of an X chromosome in a 19 year old girl. Amer. J. of Ment. Defic. 1971 (in press).

Al-Aish, M. S., Dodson, W. E., and Plato, C. C. Down's Syndrome with XYY: 48,XYY,G+. Amer. J. of Dis. of Child. 1971 (in press).

Serial No. HD-MR10/11(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Morphology Studies on Normal and Abnormal Human Chromosomes

Previous Serial Number: Same

Principal Investigator: Matti S. Al-Aish, M.A., Ph.D., Research Cytogeneticist

Other Investigators: None

Cooperating Units: National Naval Medical Center, Bethesda, Maryland

Man Years

Total	: 0.45
Professional	: 0.35
Other	: 0.10

Project Description:

Objectives: The objective of this project is to develop methods for the more precise identification of individual chromosomes. The morphology of metaphase chromosomes is the basis of the present day diagnostic procedure in cytogenetics. Many of the chromosomes do not have unique morphology and must, therefore, be placed in groups for classification. A better correlation of specific chromosome aberrations with specific clinical syndromes can be expected when methods have been established for the unequivocal identification of each chromosome.

Methods Employed: A combination of physical, chemical and enzymological methods will be employed. More specifically, this objective can be pursued by treating the chromosomes with enzymes (e.g., proteases and nucleases), by radioactive labeling techniques, and chemicals such as 5-Bromodeoxyuridine and Ca-free media. Another approach is the accurate relative measurements of each chromosome, its arm ratio, and centromeric index. In addition, a differential interference analysis of each chromosome will be done.

Major Findings: (1) Study of pronase effects on the structure of human chromosomes *in vitro* show that the longer the incubation with pronase is, the more profound the effects on the chromosome become. This study has been completed and the findings are in press. (Secondary association among the large and small acrocentric chromosomes in the human complement is controlled by homologous regions in

the short arms of these chromosomes. (3) Well formed satellites have been observed on the short arms of a submetacentric chromosome.

Significance to Biomedical Research and the Program of the Institute: The precise identification of human chromosomes is the foundation of human genetic studies. Without the accurate identification of human chromosomes, genetic mapping of chromosomes, determination of the biochemical basis of inheritance, and the description of genetic syndromes would be impossible.

Proposed Course of Project: Morphologic studies will be continued using new laboratory techniques, autoradiography, differential interference light microscopy (Nomarsky's method), and possibly electron microscopy.

Honors and Awards: None

Publications: Al-Aish, M.: Human chromosome morphology. II. Studies on the satellites and identification of the large acrocentric chromosomes. Canad. J. Genet. Cytol. 12: 160-163, 1970.

Al-Aish, M.: Human chromosome morphology. III. Pronase effects on chromosome structure in vitro. Cytologia 1971 (in press).

Serial No. HD-CD10(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics of the Kukukuku and Fore Peoples of the Kuru Region of New Guinea

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: Carlton Gajdusek, M.D.
Special Chronic Disease Studies
Richard Sorenson, M.S.
Special Chronic Disease Studies

Cooperating Unit: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.02
Professional	:	0.02
Other	:	0.00

Project Description: Complete

Honors and Awards: Results presented at the Annual Meeting of the American Association of Physical Anthropologists, Boston, Massachusetts, April 1971.

Publications: Plato, C. and Gajdusek, C.: Genetic Studies in Relation to Kuru: V. Dermatoglyphics of populations of the Eastern Highlands of New Guinea. Am. J. Hum. Genet., in press, (part of a monograph on Kuru).

Serial No. HD-CD11(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics of the Micronesians

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: D. Carleton Gajdusek, M.D.
Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke
Paul Brown, M.D., Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Cooperating Unit: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Man Years

Total	: 0.15
Professional	: 0.10
Other	: 0.05

Project Description:

Objectives: The purpose of this project is to further define the genetic composition of the people of Micronesia through complete dermatoglyphic studies, to review the literature pertaining to the worldwide distribution of various dermatoglyphic patterns, and to assess the significance of dermatoglyphics as a criterion for genetic relationship among different populations.

Methods Employed: The dermatoglyphic data were collected in conjunction with previous serological and epidemiological investigations in the area. Eight hundred sets of finger and palm prints from all the atolls of the district of Yap are presently being analyzed for the determination of the frequencies of the various finger and palm patterns and configurations.

Major Findings: This project is still in progress.

Significance to Biomedical Research and the Program of the Institute: A genetic identification of a population is helpful in order to facilitate and explain some of the results of the medical and neurological surveys by establishing certain patterns of gene migration or genetic relationships.

Proposed Course of Project: All the prints have been collected and evaluated. Statistical analysis of data is in progress. This study will be incorporated in an extensive review of the literature in an effort to establish trends in the worldwide distribution of dermatoglyphic patterns.

Honors and Awards: None

Publications: Report to be submitted for presentation at the IV International Congress of Human Genetics. September 6-11, 1971, Paris, France.

Serial No. HD-CD12(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics of Patients with Deletion of the Long Arm of the 18 Chromosome (18q- Syndrome)

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
Wladimir Wertelecki, M.D., Medical University of South Carolina

Other Investigators: Park Gerald, M.D., Boston Children's Hospital
Jerry Niswander, D.D.S., National Institute of Dental Research

Cooperating Units: Epidemiology Branch, National Cancer Institute
Division of Clinical Genetics, Boston Children's Hospital Medical Center
Human Genetics Branch, National Institute of Dental Research

Man Years

Total	0.01
Professional	0.01
Other	0.00

Project Description: Complete

Honors and Awards: Results of the study were presented at the annual meeting of the American Society of Human Genetics, San Francisco, October 1969.

Publications: Plato, C., Wertelecki, W., Gerald, P., and Niswander, J.: Dermatoglyphics in the 18q- syndrome. Ped. Res., 5: 64-69, 1971.

Serial No. HD-CD14(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Role of Xg^a Incompatibility in Fetal Loss: Family Studies

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
Felix de la Cruz, M.D., M.P.H.
Ronald Bachman, M.D., Permanente Medical Group, Oakland, California

Other Investigators: None

Cooperating Unit: Permanente Medical Group, Oakland, California

Man Years

Total	:	0.20
Professional	:	0.10
Other	:	0.10

Project Description:

Objectives: The aims of this project are (1) to confirm through family studies our earlier observations that Xg^a incompatibility may be responsible for the diminution of the expected frequency of female births to Xg^a negative mothers, and (2) to establish the degree of difference between the expected and observed frequency in female births (selection coefficient).

In our previous report, "Possible Intrauterine Interaction Between Twins Incompatible for the Xg^a Blood Group," we found a deficiency of female Xg^a positive twins. This observation held true in both the monozygotic as well as the dizygotic twins. On the other hand the observed numbers of each type of male twins were very similar to what one would expect for a sex-linked gene under random mating (no selection). Our hypothesis was that this loss of female twins is a result of mother-daughter incompatibility for this blood group which occurs when the mother is Xg^a negative ($Xg^{a-}Xg^{a-}$) and the father Xg^a positive ($Xg^{a+}Y$) and the resulting daugher Xg^a heterozygotic positive ($Xg^{a+}Xg^{a-}$). This explanation agrees very well with the results of a study by Mann et al. (in press), who arrived at the same conclusion by studying the sex ratio. The results of the aforementioned studies are based on

population expectations using the established gene frequencies of p = .65 and q = .35 for the Xg^{a+} and the Xg^{a-} alleles respectively.

Methods Employed: The present investigation will study the segregation of the Xg^a blood group in sibships. Blood will be collected from parents and all the siblings including abortuses whenever possible. Our data will be obtained partly from the Permanente Medical Group in Oakland and partly from the Naval Medical Center in Bethesda.

Major Findings: Five hundred expectant mothers have been screened for the Xg^a blood group. Of these 65 were Xg^{a-}, and 50 Xg^a weak positive. These 115 individuals plus 75 Xg^a positive controls formed the basic sample for family studies. We are presently collecting blood samples from the spouses and offspring to be used in the segregation analysis.

Significance to Biomedical Research and the Program of the Institute: The importance of this type of study to medical and child health research has been demonstrated in our earlier twin study (HD-CD16(c)). Xg^a incompatibility may be a significant factor in fetal loss, affecting only the female offspring.

Proposed Course of Project: Data collection and analysis will continue.

Honors and Awards: None

Publications: None

Serial No. HD-CD16(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Possible Intrauterine Interaction Between Twins Incompatible for the Xg^a Blood Group

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: Joseph D. Mann, M.D.
Butterworth Hospital
J. Theodore Schwartz, M.D., Head, Section on Ophthalmic Field and Developmental Research, National Eye Institute
Webster Leyshon, National Institute of Dental Research

Cooperating Units: Butterworth Hospital, Grand Rapids, Michigan
Section on Ophthalmic Field and Developmental Research, National Eye Institute

Man Years

Total	0.01
Professional	0.01
Other	0.00

Project Description: Complete

Honors and Awards: Presented as an invited paper at the First International Symposium on Twin Studies, Rome, September 1969.

Publications: Plato, C. C., Mann, J. D., Schwartz, J. T., and Leyshon, W.: The role of Xg^a blood group incompatibility in fetal loss: Twin study. In Gedda, L. (Ed.): Proc. First Int'l. Symp. Twin Studies. Published in Acta Gen. Med. et Gem. (Roma). 19: 235-237, 1970.

Serial No. HD-CD21(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Eidetic Imagery in Brain-Injured Children

Previous Serial Number: Same

Principal Investigator: Jean S. Symmes, Ph.D., Research Psychologist

Other Investigators: None

Cooperating Units: Christ Church Child Center, Bethesda, Maryland
St. Maurice's School, Potomac, Maryland

Man Years

Total	:	0.15
Professional	:	0.15
Other	:	0.00

Project Description: Complete

Honors and Awards: Paper to be read at the American Psychological Association meetings, September 1971.

Publications: Symmes, J. S.: Visual imagery in brain-injured children. Perceptual and Motor Skills (in press).

Serial No. HD-CD24(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Reading Inefficiency: Perceptual Studies

Previous Serial Number: Same

Principal Investigator: Jean S. Symmes, Ph.D., Research Psychologist

Other Investigators: Duane Alexander, M.D., Kennedy Institute, Baltimore, Maryland
Marvin Eisengart, M.D., Department of Neurology, Albert Einstein College of Medicine of Yeshiva University, New York
Judith Rapoport, M.D., Department of Psychiatry, Georgetown University

Cooperating Units: None

Man Years

Total	:	0.9
Professional	:	0.8
Other	:	0.1

Project Description:

Objectives: This study investigated patterns of perceptual functioning among children, age 7-13, of at least average intelligence who were reading well below expectation based on age and general intellectual level of functioning.

Methods Employed: The study population was screened for diagnosable medical abnormalities by a pediatrician, neurologist and child psychiatrist; EEG and tests for sensory acuity were given and had to be within the normal range for the child to be included in the study. The presence of any neurological, psychiatric or sensory abnormality, or history of any such abnormality, ruled a child out of the study population. A battery of standardized psychological tests tapping auditory, visual and cross-modality input, coding and response was administered to each of the 54 children qualifying for the study. Data has been analyzed by statistical comparison of whole group and age subgroup means and by a factor analysis of

the test battery. Individual factor scores for each child are being derived and a cluster analysis of profiles to derive clinically useful test information is planned.

Major Findings: (1) Elimination of girls. The usual sex ratio in learning disability studies is about 80% boys, and this was true of our entering population. However, the elimination of children with relevant medical antecedents or present relevant medical problems resulted in a study population of 53 boys and 1 girl. It is concluded that when other known correlates of reading disability such as neurological insult or psychiatric preoccupation are controlled there appear to be patterns of normal perceptual variation in boys that handicap them in learning to read, and that are not characteristically present in girls.

2) Lack of developmental change. Looking at the population in two-year groupings (7-9, 9-11, 11-13), the profiles of perceptual functioning remain quite constant, although tests reflecting accumulated information decline with age (e.g. WISC Information). Constant patterns of good and poor function characterize this population across age groups, which is not consonant with the commonly invoked explanation of reading difficulty as a type of developmental lag or immaturity.

3) Superiority at spatial visualization tasks. The mean scores on all tasks involving visual ordering of materials or spatial visualization are at least above average frequently exceptionally high. A factor analysis of the test data reveals a high loading on the factor primarily defined by achievement test scores of a cluster of tests that may be characterized as visual ordering of information. This population is one, then, characterized by a high correlation of visual ordering of information with achievement, by a superiority at spatial visualization tasks, and by reading significantly poorer than general intellectual functioning.

Studies of spatial visualization using twin populations and intrafamily correlations indicate (1) a generally superior performance on such tasks by males, (2) a bimodal distribution among males, and (3) a hereditary component transmitted by a x-linked recessive gene. It is suggested that inefficiency in the reading process may be associated in these children with their facility for three-dimensional encoding and processing, which may make it difficult for them to see b-p-q-d as other than one three-dimensional form. The low incidence of reading difficulty in Japan (less than 1%) where the Katakana script, used for teaching elementary reading, does not have the possibilities for spatial reversals found in English orthography, tends to support such a theoretical explanation.

Significance to Biomedical Research and the Program of the Institute: Reading disability is a significant educational problem, affecting from 5 to 30% of school populations, depending upon criteria used. Most research in this area has been done on samples of children with multiple problems and many relevant antecedents, and has looked for patterns of neurological deficit or psychiatric pathology as explanatory principles. Perhaps the principal significance of this work to biomedical research is a methodological

one, therefore. I cannot assess its significance to the program of the Institute.

Proposed Course of Project: The second phase, testing children ages 4-7, will not be done. Factor analysis of test data from the 7-13 population is completed and being written up; current statistical analysis includes derivation of factor scores, clustering to look at significant subgroupings of children and derivation of a useful clinical test battery therefrom.

Honors and Awards: Paper, "Unexpected Reading Failure," presented at the American Orthopsychiatric Association meetings, March 24, 1971.

Publications: Symmes, J. S. and Rapoport, J. L.: Unexpected reading failure. American Journal of Orthopsychiatry (in press).

Serial No. HD-CD27(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Formant Analysis of the Vowel Production of Children with Down's Syndrome

Previous Serial Number: Same

Principal Investigator: Jill Stormer, M.A., Speech Pathologist, Easter Seal Treatment Center

Other Investigator: Ira T. Lott, M.D., Clinical Associate

Cooperating Unit: Easter Seal Treatment Center, Rockville, Maryland

Man Years

Total	:	0.05
Professional	:	0.05
Other	:	0.00

Project Description:

Objectives: This study is designed to determine the central tendency of each of the first three formants in Hz for each of 10 English vowels produced by mongoloid children. Differences will be determined between (a) the experimental group of mongoloid children and a control group of retarded non-mongoloid children; (b) the experimental group and a second control group of non-retarded, non-mongoloid children; and (c) between the retarded and non-retarded control groups.

Methods Employed: Narrow band sound spectrograms and sections will be made for each vowel tested. Evaluation of differences in vowel production will be made using 1) three dimensional vowel space -- a N dimensional space calibrated in units of auditory frequency in all directions; and 2) a vowel differential -- a distance measured in Hz between any two points in a vowel space.

Major Findings: The study is in progress.

Significance to Biomedical Research and the Program of the Institute: Results from this study will be related to Dr. Ira Lott's study, currently in progress,

"Serotonin Metabolism in Down's Syndrome," and to a previous study completed at the Branch, "Speaking Fundamental Frequency in Mongolism."

Proposed Course of Project: If results of the present pilot study seem significant, the study will be expanded to include larger numbers of subjects.

Honors and Awards: None

Publications: None

Serial No. HD-CD28(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Lipid Metabolism in Down's Syndrome

Previous Serial Number: Same

Principal Investigators: Ira T. Lott, M.D., Clinical Associate
Edwin H. Kolodny, M.D., Research Associate, National Institute of Neurological Diseases and Stroke
Roscoe O. Brady, M.D., Chief, Section on Lipid Chemistry, Laboratory of Neurochemistry, National Institute of Neurological Diseases and Stroke

Other Investigators: Matti S. Al-Aish, Ph.D., Research Cytogeneticist
Duane Alexander, M.D., Kennedy Institute, Baltimore, Maryland
W. Edwin Dodson, M.D., Children's Hospital, Boston, Massachusetts

Cooperating Unit: Laboratory of Neurochemistry, Section on Lipid Chemistry, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.00
Professional	:	0.00
Other	:	0.00

Project Description:

Objectives: This study attempts characterization of lipid metabolism in patients with Down's syndrome and correlation of findings with the characteristic clinical and cytogenetic abnormalities of these patients.

Methods Employed: Erythrocytes, white blood cells, plasma, platelets, and skin fibroblasts will be subjected to the standard structural and enzymatic assays used in lipid chemistry.

Major Findings: Skin fibroblasts, leukocytes, and red blood cells did not differ in the content of gangliosides and phospholipids from those at normal

controls. It was decided that the direct study of biogenic amines in Down's Syndrome would be more informative (See Project #CD47(c), "Down's Syndrome: Cerebral monoamine turnover in patients with diminished platelet serotonin.")

Significance to Biomedical Research and the Program of the Institute: Study of lipid metabolism is of particular importance in understanding nervous system structure and function since this class of compounds constitutes over 50% of the solid weight of nervous tissue. However, to date, the study of lipid metabolism in Down's syndrome has been scanty. Recent evidence indicates that lipid metabolism may be relevant to the neoplastic tendencies and disturbances of biogenic amines seen in patients with Down's syndrome.

Proposed Course of Project: (See Project #CD47(c) "Down's Syndrome: Cerebral monoamine turnover in patients with diminished platelet serotonin.")

Honors and Awards: None

Publications: None

Serial No. HD-CD29(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Circadian Periodicity in a Child with the Prader-Willi Syndrome

Previous Serial Number: Same

Principal Investigator: Ira T. Lott, M.D., Clinical Associate

Other Investigators: David Bailey, M.D., Medical Officer, National Naval Medical Center
Ralph D. Feigin, M.D., Chief, Infectious Disease Service, St. Louis Children's Hospital, St. Louis, Missouri

Cooperating Units: Pediatric Service, National Naval Medical Center, Bethesda, Maryland
The Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri

Man Years

Total	:	0.10
Professional	:	0.10
Other	:	0.00

Project Description:

Objectives: Determination of the circadian periodicities of vital signs and selected metabolites in blood and urine of a 5 year old girl with hypothalamic disease is being made. The child's clinical picture includes mental retardation, hypotonia, hypogonadism, hypothalamic obesity, disturbance of the temperature regulating mechanism, and generalized ventricular dilatation on pneumoencephalogram with marked diminution of brain substance in the area of the hypothalamus.

Methods Employed: In a setting controlled for consistency of light, darkness, noise, and diet, the following parameters were measured at 4-hour intervals over a 48 hour period: rectal temperature; pulse, respiration, and blood pressure; urinary sodium, potassium, and chloride; urine specific gravity; and plasma cortisol and amino acids.

Major Findings: Disturbances of circadian periodicities involving temperature, pulse, urinary electrolytes, and blood amino acids were found in the above patient and have been confirmed in two other patients with the same syndrome.

Significance to Biomedical Research and the Program of the Institute: The hypothalamus has been implicated in the control of human vegetative functions such as temperature regulation and appetite. Disease involving that area of the brain is rare. The patient under study has clinical symptomatology suggestive of hypothalamic dysfunction and, in addition, has neuroradiologic evidence of decreased hypothalamic tissue. The clinical-anatomical correlation of this case lends itself particularly well to investigation of hypothalamic function and the delineation of circadian rhythm is of interest in that regard.

Proposed Course of Project: If the completed data on this patient confirm disturbance in circadian periodicity, similar observations will be undertaken on other neurological disorders involving the hypothalamus. Manuscripts are in progress.

Honors and Awards: None

Publications: None

Serial No. HD-CD30(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Blood Lactic Acid Metabolism and Urinary Catecholamines in Hyperactive Children with High Anxiety Ratings

Previous Serial Number: Same

Principal Investigators: Ira T. Lott, M.D., Clinical Associate
Duane Alexander, M.D., Kennedy Institute, Baltimore, Maryland

Other Investigators: Judith Rapoport, M.D., Department of Psychiatry, Georgetown University
Alice Abramson, M.A., Education Specialist

Cooperating Units: None

Man Years

Total	0.05
Professional	0.05
Other	0.05

Project Description:

Objectives: This study attempted to assess the feasibility and usefulness of clinically subdividing children with developmental hyperactivity into those with and without significant anxiety symptoms, and to evaluate suggestions of other investigators that L-lactate and catecholamines may be etiologic in the production of anxiety symptoms.

Methods Employed: Interview rating scales, direct playroom observations, standardized teacher's reports and actometer measurements of motor activity were used independently to classify a group of elementary school age children according to the presence of hyperactivity and anxiety. The anxious and non-anxious hyperactive children were compared with controls in regard to their blood L-lactate response to maximal exercise and their basal urinary catecholamine metabolism. Findings showing positive correlation between blood lactate, urinary catecholamines, and anxiety were to be tested for their efficacy in predicting therapeutic response to anti-hyperactivity medications.

Major Findings: The rise in whole blood L-lactate per episode of maximal exercise showed a consistent rise in all three groups and the inter-group difference was without statistical significance. Twenty-four hour urinary catecholamine excretion showed no significant difference between anxious hyperactives, non-anxious hyperactives, and controls.

Significance to Biomedical Research and the Program of the Institute: In addition to motor restlessness, hyperactivity in the school age child often presents as complaints from the teacher in the form of short attention span, labile emotional responses and anti-social behavior. These latter symptoms also characterize the child with chronic anxiety. The efficacy of anti-anxiety (phenothiazine) medications in controlling the hyperactive behavior of some children who respond adversely to sympathomimetic agents suggests that anxiety may play an etiologic role in the clinical picture of some cases of developmental hyperactivity. Of interest in recent years has been the demonstration that the rise in blood lactic acid level per second of maximum work was significantly greater for adult patients with anxiety neurosis than for controls. A controversial recent study has ascribed a causal role to lactate in the production of anxiety symptoms and has implicated catecholamines as the mediator of chronic stress in these patients. Lactate, anxiety, and catecholamines have not been previously studied in children. It was felt that the developmental hyperactivity syndrome was an appropriate condition in which to derive comparative data.

Proposed Course of Project: The negative data provided by this study suggest that lactate ion bears no significant relationship to the production of anxiety in children. The clinical classification derived to distinguish anxious from non-anxious hyperactive children seems workable and these criteria are being employed in another study testing their efficacy in predicting therapeutic response.

Honors and Awards: None

Publications: Rapoport, J. L., Lott, I. T., Alexander, D. F., and Abramson, A. U.: Urinary noradrenaline and playroom behaviour in hyperactive boys. Lancet, II, 7683, 1141, 1970.

Serial No. HD-CD32(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics of the Cypriots

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: None

Cooperating Units: None

Man Years

Total	:	0.02
Professional	:	0.02
Other	:	0.00

Project Description:

Objectives: Dermatoglyphic evaluations of two Cyprus isolates were conducted in an effort to ascertain their genetic relationship. This study represents a follow-up of an earlier hematological and serological investigation of the same isolates undertaken to establish evidence for the existence of balanced polymorphism between G-6-PD deficiency and thalassemia and malaria.

Methods Employed: Finger and palm prints were collected from unrelated residents of a coastal and a highland region of Cyprus. The prints were read and the results were evaluated statistically.

Major Findings: The dermatoglyphics of the two Cyprus isolates did not show significant differences despite long periods of isolation of the highland people and the continuous hybridization of the coastal areas.

Significance to Biomedical Research and the Program of the Institute: The study of the dermatoglyphics and other genetic markers assisted in defining the genetic composition of these isolates in a study of possible balanced polymorphism between G-6-PD deficiency and thalassemia with malaria.

Proposed Course of Project: The project is concluded.

Honors and Awards: The results were presented as an invited paper at the Symposium on Dermatoglyphics at the annual meetings of the American Society of Physical Anthropology, March 24, 1970.

Publications: Plato, C. C.: The dermatoglyphics of the Cypriots. Am. J. Phys. Anthrop., 33: 421-428, 1970.

Serial No. HD-CD33(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Polymorphism of the C Line of the Palm: A New System of Classifying the C Line Terminations

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: None

Cooperating Units: None

Man Years

Total	:	0.02
Professional	:	0.02
Other	:	0.00

Project Description:

Objectives: This study attempted to develop a more accurate method for studying the C line of the palm, and to determine possible racial, sexual and bimanual polymorphisms in the distribution of this important dermatoglyphic feature.

Methods Employed: The conventional terminations of the C line of the population studied were grouped into four modal types depending on the direction of its path. The proposed names of the four modal types are 1) Ulnar (terminations 4, 5, 6, 7), 2) Radial (terminations 9, 10, 11, 12), 3) Proximal (terminations X, $\bar{x}$ and 8), and 4) Absent, where the $\bar{c}$ triradius is absent. The literature was reviewed and the C lines were converted into the above modal types. The results were compared for racial, sexual as well as bimanual differences.

Major Findings: The suggested modal types offer an excellent tool in studying the C line termination thus providing another useful marker in genetic studies. Very distinct differences were found in the distribution of these types between the various racial groups studied. Consistent differences were also found between right and left hands.

Significance to Biomedical Research and the Program of the Institute: The finding of such distinct polymorphism with well defined racial differences provides another useful genetic marker which can be used in establishing disease associations. The persistently strong unidirectional bilateral asymmetry renders this marker especially useful in developmental problems.

Proposed Course of Project: Project completed.

Honors and Awards: Presented as an invited paper at the symposium on dermatoglyphics held in conjunction with the annual meetings of the American Society of Physical Anthropology, March 24, 1970.

Publications: Plato, C. C.: Polymorphism of the C line: A new system of classifying the C line termination. Am. J. Phys. Anthrop. 33: 413-420, 1970.

Serial No. HD-CD35(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Use of Twin Studies to Investigate the Lyon Hypothesis of X Chromosomal Inactivation with the Xg Blood Group System as Marker

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
J. Theodore Schwartz, M.D., National Eye Institute
Joseph D. Mann, M.D., Butterworth Hospital

Other Investigators: None

Cooperating Units: Field and Developmental Research, National Eye Institute
Department of Pathology, Butterworth Hospital, Grand Rapids, Michigan

Man Years

Total	:	0.10
Professional	:	0.05
Other	:	0.05

Project Description:

Objectives: This study will attempt to provide information pertinent to the Lyon hypothesis of X chromosome inactivation.

Methods Employed: Fifteen pairs of monozygotic twins have been identified as discordant for the Xg blood type. Blood specimens are being obtained from them and their parents and sibs for typing by standard methods.

Major Findings: Data collection is still is progress.

Significance to Biomedical Research and the Program of the Institute: Study of the Xg blood group marker in identical twins can provide supporting or refuting evidence for the Lyon hypothesis.

Proposed Course of Project: Arrangements are under way for data collection and blood typing of the aberrant twins and their respective relatives.

Honors and Awards: NONE Publications: NONE

Serial No. HD-CD36(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics of a Native Population of West New Guinea

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigator: Carleton Gajdusek, M.D.
Special Chronic Disease Studies, National Institute of Neurological Diseases and Stroke

Cooperating Units: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.02
Professional	:	0.02
Other	:	0.00

Project Description: Complete

Honors and Awards: None

Publications: Plato, C. C. and Gajdusek, C.: Dermatoglyphics of the Natives of Western New Guinea, submitted for publication to Human Biology.

Serial No. HD-CD 38
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Glycoprotein Metabolism in Pineal Glands in Vitro

Previous Serial Number: None

Principal Investigator: Ira T. Lott, M.D., Clinical Associate

Other Investigators: Richard H. Quarles, Ph.D., Senior Staff Fellow, Laboratory of Neurochemistry, Section on Lipid Chemistry, National Institute of Neurological Diseases and Stroke
David C. Klein, Ph.D., Senior Staff Fellow, Section on Physiological Controls, Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development

Cooperating Units: Laboratory of Neurochemistry, National Institute of Neurological Diseases and Stroke
Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development

Man Years

Total	:	0.50
Professional	:	0.50
Other	:	0.00

Project Description:

Objectives: 1) Measurement of incorporation and secretion of 3H-L-fucose labelled glycoproteins by pineal gland in organ culture; 2) separation of labelled glycoproteins of glands and media by polyacrlymide gel electrophorhesis; 3) determination of the effect of various compounds, including nor-epinephrine on the incorporation and secretion of glycoproteins by pineal gland; and 4) investigation of a physiologic function of pineal gland glycoproteins including an attempt to document a neurotropic role for secreted glycoprotein.

Methods Employed: Standard culture techniques used for in vitro studies of pineal gland from rat were empoyed. Glycoproteins were precipitated using

mixtures of trichloroacetic acid. Separation of glycoproteins, after solubilization, was accomplished by polyacrylamide gel electrophorhesis. Techniques of measurement included lipid scintillation spectrometry. Standard nerve growth factor assays were employed to measure neurotropic function.

Major Findings: Glycoproteins are actively incorporated and secreted by pineal glands in vitro. 3H-L-fucose labels only glycoproteins in this system. The polyacrlyamide gel electrophorhetic pattern of proteins within the gland is heterogenous. In contrast is a homogenous peak of secreted glycoprotein containing over 50% of all TCA insoluble radioactivity. This secreted glycoprotein is increased at the time of pineal gland innervation in the rat and there is suggestive evidence of a neurotropic function for the secreted glycoprotein.

Significance to Biomedical Research and the Program of the Institute: The pineal gland of the rat serves as a good in vitro model of neural tissue because it is derived from the roof of the 3rd ventricle and is neurally regulated by norepinephrine containing neurons whose cell bodies are located in the superior cervical ganglia. Glycoproteins are largely extracellular and in this capacity determine the specificity of the cell surface. The pineal gland in organ culture seems to be a useful model of cell:cell interactions.

Proposed Course of Project: Future work will attempt to document a functional role for the secreted glycoprotein.

Honors and Awards: Lott, I. T., Quarles, R. H., Klein, D. C. Glycoproteins are synthesized and secreted by pineal glands in vitro. To be presented by I. T. Lott at the 1971 meeting of the Fed. Amer. Soc. Exp. Biol., Chicago, April 14, 1971.

Publications: Lott, I. T., Quarles, R. H., Klein, D. C. Glycoproteins are synthesized and secreted by pineal glands in vitro. Proc. Fed. Amer. Soc. Exp. Biol. (in press).

Other publications are in progress.

Serial No. HD-CD39(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Australia Antigen in Various Types of Down's Syndrome

Previous Serial Number: None

Principal Investigator: Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Other Investigators: W. E. Dodson, M.D., Children's Hospital, Boston, Massachusetts
Earl Matthew, M.D.
Dale E. Dietzman, M.D.
David L. Madden, D.V.M.
John L. Sever, M.D.
National Institute of Neurological Diseases and Stroke

Cooperating Unit: Perinatal Research Branch, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.05
Professional	:	0.05
Other	:	0.00

Project Description:

Objectives: This preliminary study has been designed to investigate the incidence of Australia antigen (Au) in Down's syndrome patients with various karyotypes.

Methods Employed: Cytogenetic evaluation of 9 patients with Down's syndrome and 14 control patients was carried out on peripheral blood. Lymphocytes were cultured and chromosomes were karyotyped using our standard technique. In addition, skin biopsy material confirmed the findings of G/G translocation. All subjects were institutionalized, mentally retarded Negroes.

Major Findings: Trisomy 21 was found in 7, 6 of whom carried Au. Two patients with G/G translocation were identified. Neither carried Au. Only 2 out of 14 patients with normal karyotypes carried the antigen. The incidence of the Au was much higher (67%) in the Negro patients with Down's

syndrome than in their controls. This was 2-3 times higher than that reported in other populations of patients with Down's syndrome.

Significance to Biomedical Research and the Program of the Institute: It has been recently reported that the incidence of Au found in the sera of institutionalized patients with Down's syndrome has been found to be 30% and was associated with an increase of chronic hepatitis in these patients. Our data may indicate a genetic marker related to chronic viral infection in man, in this case, Australia antigen. Furthermore, a relationship between leukemia and translocation types of chromosomal aberrations has been recently reported. It will be of great importance to find out whether there is a meaningful genetic relationship between Au, leukemia and different types of Down's syndrome.

Proposed Course of Project: This pilot project indicates the extreme need for the evaluation of a larger population of patients with Down's syndrome.

Honors and Awards: None

Publications: Matthew, E. B., Dietzman, D. E., Madden, D. L., Sever, J. L., Al-Aish, M.S., and Dodson, W. E.: Possible absence of Australia antigen in G/G translocation type of Down's syndrome. Lancet I, 1971 (in press).

Serial No. HD-CD40(c)

1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cytogenetic Studies on Long Term Lymphocytes Cultures of Cystinotic, Carriers, and Normal Subjects

Previous Serial Number: None

Principal Investigators: Matti S. Al-Aish, Ph.D., Research Cytogeneticist
W. E. Dodson, M.D., Children's Hospital, Boston, Massachusetts

Other Investigators: J. D. Schulman, M.D.
K. H. Bradley
I. K. Berezesky
National Institute of Arthritis and Metabolic Diseases
P. M. Grimley, M.D., National Cancer Institute

Cooperating Units: Children's Hospital, Boston, Massachusetts
National Institute of Arthritis and Metabolic Diseases
National Cancer Institute

Man Years

Total	:	0.20
Professional	:	0.10
Other	:	0.10

Project Description:

Objectives: The objectives of this investigation are to determine chromosomal aberrations of cystinotic patients, their mothers, and normal subjects using continuous leukocytes cultures.

Methods Employed: Two cystinotic, one carrier, and four normal continuous lymphocyte lines, all approximately 6 months old, were examined cytogenetically. Ten million cells from these cultures were incubated for 72 hours at 37°C in 5 ml. T.C. 199 with phytohemagglutinin P and fetal calf serum. The cultures were processed in the usual manner for short term lymphocytes for cytogenetic analysis.

Major Findings: The modal number of chromosomes in all lines was 46. There was a marked degree of aneuploidy and an increased frequency of tetraploidy. In all lines the frequency of hypodeploidy was greater than hyperdeploidy. Structural rearrangements and breaks were seen in all cultures.

Significance to Biomedical Research and the Program of the Institute: Continuous leukocyte cultures may prove a useful tool for further studies of the causes of chromosomal abnormalities. Cytogenetic, genetic, biochemical, and morphologic investigations of such cell lines and other similar diseases are of great significance to the delineation of inherited diseases in man.

Proposed Course of Project: This project is complete. The results are being published.

Honors and Awards: None

Publications: Schulman, J. D., Bradley, K. H., Berezesky, I. K., Grimley, P. M., Dodson, W. E., and Al-Aish, M. S.: Biochemical, morphologic, and cytogenetic studies of leukocytes growing in continuous cultures from normal individuals and patients with cystinosis. Pediatric Research, 1971 (in press).

Serial No. HD-CD41(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Structure and Color of the Iris in 250 Pairs of Twins

Previous Serial Number: None

Principal Investigators: Chris C. Plato, M.S., Geneticist
J. Theodore Schwartz, M.D., National Eye Institute

Other Investigators: None

Cooperating Unit: Field and Developmental Research, National Eye Institute

Man Years

Total	:	0.25
Professional	:	0.05
Other	:	0.20

Project Description:

Objectives: (1) To establish possible association between iris structure and color and several congenital eye anomalies, and (2) to establish a scale for iris color and estimate its heretability.

Methods Employed: All of the twin pairs have undergone a thorough eye examination and evaluations. Their irises were photographed and slides were made and mounted in pairs. These slides will be used for iris structure studies. In view of technical color distortions involved in photography and slide preparation, pigment evaluation will have to be made directly by comparing the iris of each (live) eye to a predetermined set of eye color and shade model scale composed of artificial eyes.

Major Findings: Project is still in progress.

Significance to Biomedical Research and the Program of the Institute: 1) To establish possible associations between iris structure and several eye disorders, 2) to study the heretability of iris pigment and its possible relationship with eye anomalies, and 3) to offer additional criteria for the establishment of zygocity among twins.

Proposed Course of Project: To evaluate the structure and pigment of the irises and study their relationship with eye anomalies.

Honors and Awards: None

Publications: None

Serial No. HD-CD42(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Association Between Bilateral Asymmetry in Dermatoglyphics and Handedness

Previous Serial Number: None

Principal Investigators: Chris C. Plato, M.S., Geneticist
Wladimir Wertelecki, Medical University of South Carolina

Other Investigators: None

Cooperating Unit: Medical University of South Carolina

Man Years

Total	: 0.05
Professional	: 0.02
Other	: 0.03

Project Description:

Objectives: To establish the effect of handedness upon the bilateral asymmetry in dermatoglyphics and obtain better ideas about the embryological factors influencing both of these traits.

Methods Employed: Dermatoglyphics of two hundred left handed individuals and an equal number of individually matched right handed controls are being collected by standard ink method.

Major Findings: The project is still in the data collection stage.

Significance to Biomedical Research and the Program of the Institute: To understand better the embryological factors influencing the formation of dermatoglyphics and their consequent associations with clinical anomalies.

Proposed Course of Project: To finish collection and evaluation of the data.

Honors and Awards: None

Publications: None

Serial No. HD-CD43(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Dermatoglyphic Features of Normal Caucasian Americans

Previous Serial Number: None

Principal Investigators: Chris C. Plato, M.S., Geneticist
Florence Steinberg, M.S., Head, Analytical Unit, Program Statistics Branch
James Cereghino, M.D., Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Other Investigators: Bernard Fox, M.D., Perinatal Research Branch, National Institute of Neurological Diseases and Stroke
James Drorbaugh, M.D., Children's Hospital Medical Center, Boston, Massachusetts

Cooperating Units: Analytical Unit, Program Statistics Branch, National Institute of Child Health and Human Development
Collaborative and Field Research, National Institute of Neurological Diseases and Stroke
Children's Hospital Medical Center, Boston, Massachusetts

Man Years

Total	: 0.50
Professional	: 0.20
Other	: 0.30

Project Description:

Objectives: To collect dermatoglyphics (finger and palm prints) from a large sample of normal Caucasian children. The use of such a sample would be twofold: 1) To provide dermatoglyphic data from normal individuals to serve as controls for our ongoing studies pertaining to the dermatoglyphics of certain congenital anomalies, and 2) to provide the frequencies of various dermatoglyphic features in a large sample of Caucasian Americans which may serve as additional genetic markers.

Methods Employed: Finger and palm prints are being collected from 1000 children seen for a seven year evaluation at the Boston Children's Hospital

in collaboration with the Perinatal Research Branch study. Prints will be calculated by the Faurot inkless method.

Major Findings: Data collection is still in progress.

Significance to Biomedical Research and the Program of the Institute:
To provide control data for comparing the dermatoglyphics of patients with various congenital anomalies.

Proposed Course of Project: To finish collection and analysis of data.

Honors and Awards: None

Publications: None

Serial No. HD- CD44(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphic Features of Normal Black Americans

Previous Serial Number: None

Principal Investigators: Florence Steinberg, M.S., Head, Analytical Unit, Program Statistics Branch
James Cereghino, M.D., Collaborative and Field Research, National Institute of Neurological Diseases and Stroke
Chris C. Plato, M.S., Geneticist

Other Investigator: Dr. Jane Hardy, Johns Hopkins University

Cooperating Units: Collaborative and Field Research, National Institute of Neurological Diseases and Stroke
Johns Hopkins University, Baltimore, Maryland

Man Years

Total	: 0.25
Professional	: 0.05
Other	: 0.20

Project Description:

Objectives: To collect and evaluate the dermatoglyphics of a large sample of Black Americans. Such a sample will be used as controls for our ongoing studies on the dermatoglyphics of various congenital anomalies.

Methods Employed: Finger and palm prints were collected from over five hundred Black Americans from the Baltimore area through the standard ink method.

Major Findings: Data are presently being evaluated.

Significance to Biomedical Research and the Program of the Institute: To offer appropriate controls for the study of the dermatoglyphics and their developmental involvement in several congenital anomalies.

Proposed Course of Project: To finish the statistical analyses of the data and prepare a manuscript for publication.

Honors and Awards: None

Publications: None

Serial No. HD-CD45(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics of Leukemia Patients and Their Relatives

Previous Serial Number: None

Principal Investigators: Chris C. Plato, M.S., Geneticist
Wladimir Wertelecki, M.D., Department of Pediatrics, University of South Carolina

Other Investigators: Jerry D. Niswander, D.D.S., Department of Human Genetics, National Institute of Dental Research
J. F. Fraumeni, Epidemiology Branch, National Cancer Institute

Cooperating Units: Department of Pediatrics, University of South Carolina
Epidemiology Branch, National Cancer Institute
Human Genetics Branch, National Institute of Dental Research

Man Years

Total	:	0.35
Professional	:	0.20
Other	:	0.15

Project Description:

Objectives: This study represents a follow-up effort to establish possible associations between known dermatoglyphic markers and the various forms of leukemia.

Methods Employed: Dermatoglyphics (finger and palm prints) have been collected from 130 leukemia patients and as many of their first degree relatives as possible. All prints have been read and evaluated completely. A new computer program has been developed by the authors for data storage, retrieval and analysis.

Major Findings: An excess of whorls among the leukemia male patients. This agrees with the differences observed in the birth weight of the male patients. This becomes significant since it might relate to differences in the develop-

mental stage of leukemia between the male and female patients. The patients of both sexes showed higher frequencies of simian and Sydney palm lines.

Significance to Biomedical Research and the Program of the Institute: Demonstration of dermatoglyphic markers in leukemia would add a new tool to study of this disease.

Proposed Course of Project: Data on the families is being programmed for computer analysis. A report will be prepared for publication.

Honors and Awards: Reports to be presented at the IV International Congress of Human Genetics, Paris, France, September, 1971: 1) Wertelecki, W., Plato, C. C., Fraumeni, J. F., and Niswander, J. D.: Dermatoglyphics of patients with acute lymphatic leukemia and their first degree relatives, 2) Wertelecki, W. and Plato, C. C.: A computer method for recording, storing and analyzing dermatoglyphic data.

Publications: None

Serial No. HD-CD46(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics of the Natives of Papua, New Guinea

Previous Serial Number: None

Principal Investigators: Chris C. Plato, M.S., Geneticist
Carlton Gajdusek, M.D., National Institute of Neurological Diseases and Stroke

Other Investigators: None

Cooperating Unit: Special Chronic Disease Studies, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.05
Professional	:	0.01
Other	:	0.04

Project Description:

Objectives: To evaluate the dermatoglyphics of these people as a part of a multidisciplinary investigation to identify the frequencies of the various genetic markers of the aboriginal populations through the world.

Methods Employed: Collect finger and palm prints from three areas of Papua and evaluate them in detail.

Significance to Biomedical Research and the Program of the Institute: The study of genetic markers of the few remaining primitive populations will allow scientists later on to evaluate the effects of western culture and environmental pollution upon these societies.

Proposed Course of Project: To evaluate the collected data.

Honors and Awards: None

Publications: None

Serial No. HD-CD47(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Down's Syndrome: Cerebral Monoamine Turnover in Patients with Diminished Platelet Serotonin

Previous Serial Number: None

Principal Investigator: Ira T. Lott, M.D., Clinical Associate

Other Investigators: Dennis L. Murphy, M.D., Head, Section on Psychiatry Laboratory of Clinical Science, National Institute of Mental Health
Thomas N. Chase, M.D., Chief, Section on Experimental Therapeutics, Laboratory of Clinical Science, National Institute of Mental Health
Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Cooperating Units: Laboratory of Clinical Science, National Institute of Mental Health
2-West Neuromedical Service, Clinical Center

Man Years

Total	:	0.30
Professional	:	0.30
Other	:	0.00

Project Description:

Objectives: To ascertain whether low concentration of serotonin (5-HT) in the platelets of Down's syndrome patients is associated with an abnormality in the turnover of this monoamine within the central nervous system.

Methods Employed: I. Platelets isolated from venous blood of trisomy 21 patients were examined for endogenous serotonin content, rate of uptake of (14C-) serotonin in vitro, binding of the amine, activity of monoamine oxidase, and effect of various pharmacologic agents on the uptake and binding of 5HT.

II. Patients with Trisomy 21 over 18 years of age underwent lumbar puncture at intervals during the oral administration of probenecid.

Major Findings: I. Endogenous 5HT in platelets was reduced by 61% in Down's syndrome patients compared to several control groups. The low concentration of serotonin in platelets was associated with a defective uptake of this amine. Monoamine oxidase activity was normal.

II. Steady state levels of 5-HIAA (the principle metabolite of 5HT) in lumbar CSF just prior to probenecid administration did not differ from control subjects. Following probenecid administration, the concentration of 5-HIAA in CSF increased to 3 times baseline values in both patients and controls.

Significance to Biomedical Research and the Program of the Institute: The human blood platelet has been described as a model of the andrenergic neuron. The low concentration of serotonin in the platelets of Down's syndrome patients has suggested to some observers that a defect in cerebral monoamine transport or metabolism may attend this disorder. The findings of the above study suggest that diminished platelet 5HT levels in Down's syndrome are not associated with alterations in the cerebral metabolism or transport of 5HT.

Proposed Course of Project: An abnormality in the content of ATP in Down's syndrome platelets would explain the defective transport and binding of serotonin. Such an abnormality has been recently described and might form the basis of a future study. Publications are in progress.

Honors and Awards: Abstract to be presented at the 23rd Annual Meeting of the American Academy of Neurology, New York City, May 1, 1970. I. T. Lott, D. L. Murphy, and T. N. Chase. "Down's Syndrome: Cerebral monoamine turnover in patients with diminished platelet serotonin."

Publications: Lott, I. T., Murphy, D. L., and Chase, T. N.: Down's Syndrome: Cerebral monoamine turnover in patients with diminished platelet serotonin. Neurology, 1971 (in press).

Serial No. HD-CD48(c)

1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970, through June 30, 1971

Project Title: Dermatoglyphics and Cleft Palate: Familial Investigation

Previous Serial Number: None

Principal Investigators: Chris C. Plato, M.S., Geneticist
Wladimir Wertelecki, M.D., Department of Pediatrics, University of South Carolina
J. D. Niswander, D.D.S., National Institute of Dental Research

Other Investigators: None

Cooperating Units: Human Genetics Branch, National Institute of Dental Research
Department of Pediatrics, University of South Carolina

Man Years

Total	:	0.25
Professional	:	0.15
Other	:	0.10

Project Description:

Objectives: This study will look for possible associations between dermatoglyphic markers and the various forms of cleft palate.

Methods Employed: Finger and palm prints were collected from cleft palate patients, their parents, and sibs from over 100 families. An equal number individually matched controls were selected and their dermatoglyphics were also obtained along with those of their first degree relatives. The results will be statistically compared for possible differences between (1) individual patients and controls, (2) patients and their first degree relatives, (3) the patient families and controls, and (4) bilateral differences will also be investigated.

Major Findings: The project is still in progress.

Significance to Biomedical Research and the Program of the Institute: Identification of dermatoglyphic markers associated with cleft palate would be of possible assistance in understanding the genetics and origin of this malformation.

Proposed Course of Project: The dermatoglyphics of all of the controls and their relatives have been completed. Most of the patient data have also been evaluated.

Honors and Awards: None

Publications: None

Serial No. HD-CD49(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Dermatoglyphics of Patients with Congenital Heart Disease

Previous Serial Number: None

Principal Investigators: Chris C. Plato, M.S., Geneticist
Wladimir Wertelecki, M.D., Medical University of South Carolina

Other Investigators: None

Cooperating Unit: Medical University of South Carolina

Man Years

Total	:	0.05
Professional	:	0.02
Other	:	0.03

Project Description:

Objectives: To establish possible associations between various dermatoglyphic traits and congenital heart disease.

Methods Employed: Finger and palm prints were collected by the standard ink method from eighty patients with congenital heart disease and are presently being evaluated.

Major Findings: Project in progress.

Significance to Biomedical Research and the Program of the Institute: To provide possible dermatoglyphic markers in an effort to understand the etiology and possible inheritance of this disease.

Proposed Course of Project: To evaluate and analyze the data. If warranted family data will be collected.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1970 through June 30, 1971

Gerontology Research Center
Office of the Chief

During the year progress has been made in understanding the basic mechanisms of aging. In the area of molecular biology, new polymers of nucleotides have been designed, synthesized and evaluated for their selective uptake into cells and induction of viral protection. A new complex of polyinosinic acid and poly(1-vinylcytosine) has shown high interferon activity and it is postulated that the high anti-viral activity is related to the reduction of the charge/mass ratio and to the existence of this complex in an aggregated state. This finding gives great potential for introducing other compounds into cells by forming complexes and studying their effects on development and aging.

Studies have shown that, with increasing age, cells in a variety of animals (rotifers, crayfish, mice, rats) show an increased accumulation of lysosomes and lysosomal enzymes which break down cytoplasmic substances. Similar increases in lysosomal enzymes have been found in cells of organs undergoing hormone induced involution. Thus further evidence has accumulated to indicate the importance of lysosomes in cell loss, which is associated with aging.

After experimental surgical wounds, young animals (rats) produce more scar tissue than do the old. Furthermore, the tissue produced by young animals is more compliant. This indicates the presence of cellular mechanisms in the older animal which induce it to form tissue that is more similar to that found in old than young animals, i.e., increased stiffness.

The decrease in maximum O_2 uptake of mitochondria isolated from insect flight muscle observed in old flies is localized in the respiratory chain. Ultrastructural studies showed a unique degradation of mitochondrial cristae in mitochondria from old flies.

A non-invasive technique for the assessment of ventricular function by computation of systolic time intervals has been applied to 333 normal men aged 20 to 93. In subjects with no clinical evidence of heart disease, the total ejection time and the pre-ejection phase increased to a maximum at age 60. After age 60 both variables declined. Since these increasing values may reflect a decrement in ventricular function with age, the failure of average values to increase after age 60 may be a reflection of selective mortality--subjects with high values may have higher mortality rates in later life so that cross-sectional data may not show a further increase after age 60. The longitudinal follow-up of individual subjects will eventually answer this important question.

Using glucose-1-^{14}C in tracer studies it was shown that with the infusion of insulin the decrease in hepatic glucose production and increase in peripheral glucose utilization was the same in old and young subjects.

Furthermore, metabolic recycling of glucose did not differ with age. The conclusion that aging reduces β cell sensitivity is further strengthened.

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The administration of the Gerontology Research Center was made easier this year with the assignment of a full time Administrative Officer. Services to and for the research programs were further improved. For example, more efficient servicing of the mechanical/electrical equipment in the building will result from the Center linking up with the Baltimore City Hospitals computerized program for periodic maintenance of equipment.

Research Services

Through the efforts of the Technical Development Section, the digital computer system is now operating effectively. Classes in both Fortran and Assembly languages were offered to staff of the Center so that an increasing number of investigators are now effectively using this resource. The Section modified a surplus plotter and added this to the computer to permit graphic presentations of data. The computer has also been programmed to administer expanded problem solving tests. A major program was developed which enables the computer system's remote teletypes to be used as programmable calculators on a time-shared basis. Thus, a capability now exists whereby simple, but time consuming, calculations can be done on the computer by staff with a minimum of training.

This Section completed a system for the Section on Human Learning which can be used to administer short-term memory tests. This system controls the presentation of audio information to the subject and records the nature and time of response. Major modifications were made for both the paired-associate testing and the time judgment equipment for this same section. In the Human Performance Section, an existing tonometer was built into a system to reflect carotid artery patency in intra-orbital tension. Equipment was assembled in the same section for measurement of systolic time intervals as an index of left ventricular function in human subjects.

The Photography and Arts Section dealt with increasing demands for visual communication services by staff. The total work load showed a significant rise over last year. Forty-two Center scientists and three guest scientists requested 356 drawings (up 18%), 899 photographic negatives (down 33%), 4,731 photographic prints (up 18%), and 2,957 projection slides (up 93%). This Section also processed 148 rolls of film in support of projects underway in the Laboratory of Cellular and Comparative Physiology.

Development of the new rat colony in the Animal Resources Facility is proceeding on schedule. The first animals from this "clean" colony will be issued to Center scientists in October 1971. Currently over 7,700 rats are being maintained in this colony. The cumulative mortality-morbidity index for these animals over the past 21 months is 6.10%. The old rat colony of about 3,500 animals will be phased out by October 1971. In

addition to the rat colonies, the ARF houses 36 dogs, 3 rhesus monkeys, 10 rabbits, and a small mouse colony for use of Center staff.

Progress during the year included installation of a new vacuum line for disposing of animal waste. Although this system still needs some adjustment, it is now handling disposal of about 75% of the waste produced by the rat colonies.

Information Services. The Center Chief and Dr. Reubin Andres appeared on "You and Everyone's Future," shown twice over WRC-TV (Washington) in early 1971. The Information Officer wrote the working script and helped plan this HEW-produced program. Other television contacts included those with Westinghouse Broadcasting, West German Television, National Educational Television, WJZ-TV (Baltimore), and the Maryland Center for Public Broadcasting. Popular articles on the Center appeared in the New York Times, Saturday Review, True, Family Weekly, Good Housekeeping, the Washington Post, the Sun, and the News-American (both in Baltimore). MD Medical News, Today's Health, Modern Maturity, Health, and Geriatric Focus were some of the specialized publications requesting information.

The number of Center tours increased to 38 this year from 24 last year (up 58%), and public inquiries rose to 193 from 162 (up 19%). Additionally, special Baltimore itineraries were arranged for scientists from Belgium and Wales. Other activities included preparation of an article on Laboratory of Cellular and Comparative Physiology studies for Zeiss Information, and editing or rewriting a number of scientific papers for publication in The Gerontologist.

In addition to providing regular services to Center investigators, the Library staff continued to index world literature relating to gerontology. This past year over 3,000 references were identified and published in quarterly issues of the Journal of Gerontology.

Collaborative Guest Scientist Program

During the past year eight guest scientist programs utilized the Center's resources. These programs are staffed by 19 scientists and 12 technicians at present.

Cholera toxin was administered intravenously to dogs, mice, and rats in order to study non-intestinal effects in intact animals. Biochemical determinations on serum and specific tissues (liver, blood platelets) add to the growing evidence that cholera toxin alters cell function by activating adenyl cyclase activity. The effect of the toxin is delayed in onset, prolonged in duration, and active on a variety of cell types. This suggests that cholera toxin may prove a unique tool for the manipulation and study of adenyl cyclase-cAMP mediated processes. It may also be extremely useful for studying the functions of aging cells and organs. (Dr. Carpenter).

In another study, dogs immunized with cholera toxin plus adjuvant were protected against living vibrio challenge for over 10 months after

immunization. This protection correlated with serum antitoxin titers. Jejunal response to the toxin was significantly reduced following passive transfer of antitoxic serum. Intestinal fluid from convalescent cholera and non-cholera patients contained considerable toxin-neutralizing capacity, not present in patients with the acute disease or in normal controls. This material neutralized both cholera toxin and E. coli enterotoxin. In at least half of the patients it retained full activity after 30 minutes boiling, suggesting it was not a protein antibody. It is known that acute infectious diarrheas decrease in incidence with increasing age but the mechanisms involved are not clear. Acquired antibacterial immunity may play a role or antitoxic immunity might occur. The demonstration of an acquired heat-stable antitoxic material suggests that non-immune mechanisms may also be involved. Further study of these mechanisms may help explain the means by which the small bowel is usually maintained nearly bacteria-free. (Dr. Carpenter).

In a related study the way in which purified cholera toxin interacts with cells is being investigated. Epididymal fat pads from rats are incubated in collagenase and passed through silk netting. The resulting isolated fat cell suspension is then incubated under various conditions to explore the nature of cell-toxin interactions. The cholera toxin stimulated the breakdown of triglyceride to glycerol and free fatty acids. This reaction does not depend on protein synthesis but on the effect of calcium, magnesium, and potassium ions. The lipolytic stimulating effect is blocked with specific antisera permitting the system to be used to titrate both toxin and antitoxin. The age of rats used to obtain the lipocytes is important since the older rats showed a higher threshold to the toxin effects. Adenyl cyclase was also stimulated and cyclic AMP levels raised by preincubating fat cells with toxin. (Dr. Greenough).

In other work, the same investigators found a novel secretory process in the small bowel which became evident as a consequence of raised cyclic AMP levels. They found this when intestinal mucosa was taken from rabbits or human surgical specimens and mounted in a lucite chamber after stripping off muscular and serosal layers. They measured electrical gradients potentiometrically and fluxes of sodium and chloride isotopically in studying the action of cholera toxin on gut mucosal ion transport processes. The findings were as follows: (1) rabbit mucosal function is affected by structural abnormality; (2) rabbit ileum responds to cholera toxin, theophylline and cyclic AMP by reducing net sodium ion adsorption and by secreting chloride ion; and, (3) human ileum also showed similar effects when exposed to cholera toxin. Cholera toxin apparently triggers the novel secretory process noted. The normal physiologic control mechanism, however, is still unknown. Determination of this new physiologic function would aid considerably in understanding intestinal function at all ages. (Dr. Greenough).

Guest investigators are utilizing hormone-induced involution in endocrine-related organs as a model for the study of senile involution. Castration in male rats is followed by marked involution of sex accessory organs--prostate, seminal vesicle, and epididymis, due to withdrawal of circulating androgens. This also occurs in hypophysectomized rats due to

suppression of interstitial cell stimulating hormone. Cytochemical-electron-microscopial studies have shown an apparent increase in acid phosphatase activity, especially concentrated in dense bodies and in autophagic vacuoles. This activity was present in the epithelial cells and, to a lesser extent, in smooth muscle fibers. On the other hand, biochemical analysis showed a decrease in various acid hydrolases in the sex accessory organs of castrated or hypophysectomized rats. This research indicates that, during hormonally induced involution, apparent increases in acid hydrolytic activities reflect a process of selective retention and redistribution of such enzymes--not new enzyme synthesis. This does not, however, rule out active participation of lysosomal hydrolases in involution since the enzymes redistributed in the cytoplasm appear to be actively engaged in the breakdown of cytoplasmic components within autophagic vacuoles. (Dr. Brandes).

The morphologic expression of the graft versus host reaction has been tentatively described from the wealth of autopsy and surgical material available from patients receiving bone marrow transplantation. The graft-host reaction is caused by the interaction of donor immunologically active cells with host organs, primarily the skin, liver, and gastrointestinal tract. There is, on the other hand, confusion in the literature concerning these morphologic changes with alterations induced by chemotherapy and radiation. Guest scientists have designed a series of experiments to reproduce in animals the human morphologic findings and to elucidate the mechanism by which immunologically active cells act on recipient cells. This latter is important not only for gaining a better understanding of the graft versus host reaction mechanism but in explaining cellular immunity in general. Four groups of rats were studied. One group received high doses of Cytoxan without transplantation; the second received high doses of Cytoxan and spleen cell transplants from syngeneic donors; the third received Cytoxan and spleen cell transplants from allogeneic donors exhibiting only minor differences in histocompatibility antigen; and, the fourth group received Cytoxan with transplantation of spleen from allogeneic donors exhibiting major differences in transplantation antigens. Autopsy and examination of sections by light and electron microscopy indicated changes identical with those seen in humans for the rats from the last group (those receiving Cytoxan and spleen transplants from donors exhibiting major differences in transplantation antigens). These were the animals whose situation was nearest that of the human material studied previously. Further electron microscopy studies are underway to determine the interaction between transplanted immunologically active lymphoid cells and recipient organs. (Dr. Slavin).

Recent research indicates that while amyloid fibrils are usually resistant to degradation, treatment with fibril antisera results in active fibril phagocytosis. The drug D penicillamine has been studied as a possible therapeutic agent for amyloidosis. Amyloidosis was induced in white, female New Zealand rabbits by the subcutaneous injection of sodium caseinate (5 ml. of a 10% suspension biweekly). Animals treated with D penicillamine received 75 mg. per kilogram body weight every other day. Four groups of rabbits were studied. One received caseinate only, another penicillamine alone, another penicillamine and sodium caseinate simultaneously. The

fourth group received sodium caseinate until they developed amyloidosis and then D penicillamine was added to the regimen. The early results suggest that penicillamine has no demonstrable effect on either amyloid accumulation or removal. However, splenic amyloid tended to regress after 18 to 22 weeks of sodium caseinate administration. Furthermore, at this time there was an accumulation of multinucleated giant cells within the spleen and light microscopy suggested that active phagocytosis was taking place. These studies suggest that spontaneous regression of amyloid may involve an immune mechanism of some sort. Spontaneous amyloid regression in humans is a rare, but well documented, phenomenon. This regression might be accelerated by appropriate immunologic therapy. (Dr. Wright).

The same investigators are studying the relationship of cerebral amyloidosis to cerebral function. Cerebral amyloidosis, in the form of senile plaques and meningovascular involvement, is common in aged persons. The present work explored the possible relationship of cerebral amyloidosis to disturbed mental function in the aged. This retrospective study involved 200 patients over age 60 dying at several large community hospitals in Buffalo, New York, and in Baltimore, Maryland. All patients in whom assessment of mental function was questionable were excluded. Results to date indicate that the frequency of cerebral amyloidosis rapidly increased after 75 years of age. Approximately 90% of patients over 80 years of age exhibited this lesion. Initial correlations suggested that the accumulation of cerebral amyloid might be associated with the development of the so-called "chronic brain syndrome."

Studies on the effects of prostaglandins on electrical and motor activity in isolated colon muscle strips from humans and dogs have shown that prostaglandins significantly increase the frequency and amplitude of electrical slow waves associated with augmented "tone" in longitudinal muscle of the gut. They also increase spike potentials with strong phasic contractions. Hyoscine and atropine administered before or after prostaglandins decrease both tonic and phasic contractions, while hexamethonium has no effect. In circular muscle some prostaglandins produce contractions, while others cause relaxation. These results suggest that prostaglandins act on postganglionic cholinergic receptors since hexamethonium, a ganglionic blocker, does not inhibit prostaglandin induced responses while hyoscine, a postganglionic blocker, does. (Dr. Schuster).

In related research, this investigator is trying to define the effect of alcohol and or malnutrition on secretion and water absorption, glucose, and electrolytes in the jejunum and ileum. The effects noted are then correlated with motor changes in the jejunum and ileum and with folic acid absorption. Male alcoholic patients, between 20 and 60 years of age without evidence of chronic gastrointestinal or liver disease, are utilized in this research. This work has demonstrated that folate absorption is impaired in alcoholics. However, this is not related to alcohol intake per se since folate absorption returns to normal, despite alcohol consumption, if adequate dietary intake is assured. The diarrhea associated with the alcohol consumption does not appear to be related to secretions since patients have so far not demonstrated secretion during these studies. (Dr. Schuster).

These studies are providing physiologic information related to the

mechanisms involved in continence and the altered motility in constipation and diarrhea. Since constipation and incontinence are found with increasing frequency in older people, such studies may help separate age-associated changes from those that result from constipation alone. The secretory studies, while not primarily directed toward the effects of aging on secretory status, can provide basic information on folate absorption. This information can then be used to learn more about the hematologic disorders occurring with age.

One guest investigator is studying growth limitation and regeneration in the common barnyard or zebra earthworm Eisenia foetida. This species is slightly over 90 segments in length. When posterior segments are cut off the worm regenerates the same number of segments as were removed. Regeneration of posterior segments was not influenced by surgical removal of brain or ventral nerve cord. The number of segments regenerated depended on the original position of the segment in the intact worm. Histologic examination of regenerating ends showed that each new segment represents only two cell divisions of the lead cells (teloblasts) at the growing tip. Thus the number of cell generations from the start of regeneration is about 150-250. (Dr. Moment).

In another experiment, this investigator studied the effects of irradiation in limiting growth. There has been much work on X-ray effects on the regeneration of various types of worms, but no one has looked at earthworms where there is a fixed and accurately known amount of regeneration to be expected. Eight groups of 10 or more earthworms each were subjected to radiation of 10,000, 20,000, or 30,000 rads over accurately determined portions of their length and regeneration was observed following amputation at the level of the 50th segment from which the amount of regeneration to be expected is known.

Preliminary results indicate the following: (1) Total body irradiation of 20,000 rads completely and permanently inhibits regeneration; (2) Shielding the first 40 segments and irradiating segments 41 through to the worm's end prevents any regeneration in all cases up to the end of the third week when controls cut at the level of the 50th segment had finished the addition of new segments; (3) Conversely, irradiating the first 40 segments and shielding the others resulted in normal regeneration; and (4) By the end of the seventh week all but four worms shielded up to the 40 segments and irradiated the rest of their length began to regenerate and some had 20 segments regenerated. It is clear from the third finding that the 10 segments adjacent to the cut surface have the capacity not only to regenerate but to form the correct number of segments independently of any embryonic reserve cells or any "information" that could be destroyed by X-rays located in the 40 exterior segments. (Dr. Moment).

Last fall, Dr. Walter C. Stolov and co-workers from the University of Washington, Seattle, spent time at the Center studying the effects of denervation on age changes in the collagen content of rat gastrocnemious muscle. They studied two groups of Wistar rats--31 "old" animals with an average age of 705 days (Group I) and 39 "young" rats averaging 342 days of age (Group II). Three weight matched subgroups were formed within the

young and old groups. A subgroup from each age cohort was sacrificed at the beginning of the experiment. Two other subgroups had one leg denervated, and two subgroups were left undisturbed. After 13 weeks the animals were sacrificed and a comparison was made of muscle weight and hydroxyproline content between the undenervated limb muscles of subgroups having one leg denervated and the muscles of undisturbed animals. Data indicated that denervating one limb did not alter muscle weight and hydroxyproline content of the undenervated muscle. Comparisons of early and late sacrificed young and old normal animals indicated the following: (1) Total muscle collagen increased with age to a maximum at about 19-22 months, then decreased; (2) Total muscle mass also increased to a maximum at around 15-19 months, then decreased but at a faster rate than collagen; (3) Collagen concentration increased with growth, leveling off between 15-23 months, then markedly increased with old age. Denervation resulted in a slower accumulation rate of total collagen in young animals and a faster fall in total collagen in the old. An increased rate of degradation induced by denervation probably accounts for this finding.

Intramural Program

In the Laboratory of Molecular Aging new polymers of nucleotides and their analogues have been designed, synthesized and evaluated with respect to their selective uptake into cells with the aim of conferring viral protection and potentially of affecting life span of cells. Structural changes in the potent interferon inducer poly I - poly C that modify the polymer backbone substantially, such as loops on 2' $\rightarrow$ 5' phosphodiester bonds, lead to decreased antiviral activity. However, the complex of polyinosinic acid and poly(1-vinylcytosine) showed high interferon induction activity. It is postulated that the high antiviral activity is related to the reduction of the charge mass ratio of the molecule and to the existence of this complex in an aggregated state.

It has been demonstrated that elastin fibers in the lungs and blood vessels of rats change qualitatively with age. The lamellar and interlamellar elastin fibers in aortas of rats (12-36 months) are more susceptible to hydrolysis by purified porcine pancreatic elastoproteinase than are fibers of animals under one year of age. Elastic layers around the alveoli and small blood vessels in old rat lung are also more readily digested. Elastic fibers of the aorta, however, become more resistant to hydrolysis by elastomucases while the rat ages from 1-24 months. When biochemical properties of aortal rings were examined after treatment with elastolytic enzymes, their strength was found to increase as a function of age.

The cellular mechanisms involved in the decrease in muscular work performance with aging are investigated by studying insects. Using isolated flight muscle mitochondria from senescent flies, investigators found a significantly lower State 3 rate of respiration (the maximal O_2 uptake rate obtainable in the presence of excess substrate, ADP, and oxygen) in old fly mitochondria than in that from mature flies. Neither the "resting" State 4 rate nor the ADP O ratio is altered by age. The decreased State 3 rate is obtained with two physiological substrates of this muscle, alpha-glycero-

phosphate and pyruvate + proline. This indicates that the decrement in oxidative capacity is localized in the respiration chain. Ultrastructure studies showed a unique degradation in mitochondrial cristae in flight muscle of old flies.

Using the healing surgical wound to examine aging mechanisms of soft connective tissues has shown that young rats (aged 8 months) produce considerably more scar tissue after incision than do old rats (aged 18 months). This is apparent in that the scar tissue from young animals has a greater breaking strength than scar tissue from old animals. Connective tissue produced by the younger rats is more compliant than that produced by the older animals. This points to cellular mechanisms in the older rats that cause their regenerated tissues to have increased stiffness--a characteristic associated with aging in connective tissue.

Histochemical studies in the Laboratory of Cellular and Comparative Physiology strongly suggest that inclusion bodies (cellular organelles) which increase in concentration in several organs of old rotifers are some form of lysosomes. Earlier work showed that the age-dependent distribution of two lysosomal enzymes, acid phosphatase and β-glucuronidase, was consistent with age-dependent alterations in inclusion body concentrations. A third lysosomal enzyme, E600 resistant esterase, studied histochemically, was shown to increase its activity in the stomach, ovaries, and dorsal gastric gland of old rotifers. This pattern is consistent with the age-dependent changes in inclusion body concentration noted previously.

There is scant knowledge about the activities of highly differentiated, non-renewable cells as they relate to changes in tissue and organ function with age. A recently completed ultrastructural study of left ventricle myocardial cells of rats ranging from 6 to 33 months of age indicated that marked changes in fine structure begin to occur at about 18 months and continue through 33 months. The changes included increases in the numbers of residual bodies, increases in primary lysosomes in the mitochondrial cones at the nuclear poles and in mitochondrial rows between myofilaments; lysosomal degradation of mitochondria; increased numbers and changes in the nature of lipid droplets; and partial or complete degradation of lipid droplets by lysosomal activity and auto-oxidation. These studies indicate that degradation of mitochondria to residual bodies by lysosomal activity occurs with aging.

Poikilothermic animals have shortened life spans when exposed to high temperature. Studies on the hydranth, Campanularia flexuosa, have shown another interesting side to the longevity of these organisms. That is, in spite of constant laboratory environmental conditions, there are seasonal fluctuations in life span that seem related to changes in the environmental temperature of their natural habitat. For example, the shortest laboratory life span occurred when the water temperature at Woods Hole (the natural habitat) was highest. The longest life span occurred when the Woods Hole water temperature was lowest.

A previously unrecognized nucleotide has been isolated from the erythrocytes of cold-exposed and hibernating hamsters. The concentration

of this nucleotide increases during hibernation. While its spectral characteristics resemble inosine phosphate, thin layer chromatography and other experiments showed it was not identical with IMP, IDP, or ITP. Inosine and adenosine used as supplements to blood preservatives increase erythrocyte viability in vitro. Therefore, further study is needed to find out whether this inosine-like compound accounts for the longer erythrocyte life span noted in hibernating hamsters as compared to control animals.

Investigators in the Clinical Physiology Branch have continued testing approximately 600 normal male subjects in the Longitudinal Study of Aging. Recruitment of new subjects in the program has been limited to young and very old subjects to supply replacements.

Advances have been made in data retrieval and computer programming so that cross-sectional and longitudinal analyses of multiple variables can now be provided for investigators. Serial observations on selected variables made in the same subjects from 1960 to 1969 are now being analyzed.

A programmed stress test (treadmill exercise) designed to increase sensitivity of the electrocardiographic diagnosis of latent or potential coronary artery disease has been performed on 345 longitudinal subjects. The results, so far, indicate that more positive responses are obtained with this controlled treadmill regimen than with the conventional Master's two-step test. Presumably this shows increased sensitivity in the detection of early coronary artery disease, although long-term follow-up is still necessary to rule out the possibility of decreased specificity (false postions) on the stress test. Eighty-eight subjects have now had two stress tests and the results on paired tests are similar.

A new test for the assessment of ventricular function in humans, by the measurement of systolic time intervals, has been applied to 333 normal subjects from the Longitudinal Study group. Average values for total ejection time and the pre-ejection phase lengthen with increasing age up to the age of 60, indicating a reduction in ventricular function with increasing age. After this age both of these variables tend to diminish. The failure of average values to increase after age 60 may be due to selective mortality. Longitudinal follow-up studies on the same subjects will be necessary.

This year more detailed evaluation of cerebrovascular status and differential supra-orbital temperature measurements have been added to the longitudinal protocol. Supra-orbital skin is supplied by branches of the carotid artery. The potential value of these measurements in screening for cerebrovascular disease will be tested during future follow-up work with longitudinal volunteers.

Using chronic catheterization of the rat right atrium and aorta, blood pressure and cardiac function have been measured in unanesthetized and fairly unrestrained animals. No differences in cardiac function were found in 13 rats aged 24 months or in 18 rats aged 12 months . These results differ from those obtained from isolated heart or heart lung preparations in which the rat hearts are severely stressed, in fact, dying. It is now feasible

to undertake detailed pharmacologic studies in intact animals on the control factors in cardiac function that may relate to age.

Glucose-clamp studies in prediabetics were done this year in collaboration with New York and Boston investigators. Seven subjects with normal glucose tolerance tests were studied. The subjects were either offspring of diabetic parents, or an identical twin of a diabetic sibling. The major defect in these subjects was a diminution in the rapid insulin response occurring during the first few minutes after induction of a new steady state of hyperglycemia. Six non-obese diabetic subjects also showed a definite diminution in this rapid insulin peak. These people, however, also showed a lower than normal insulin concentration all through the two-hour hyperglycemic plateau period.

Tracer studies, using glucose-1-^{14}C infusions, have been performed in young and old male subjects. Under basal conditions glucose production and utilization averaged 1.8 mg. glucose per Kg. body weight per minute in both young and old. When insulin was infused to give a steady blood level of 100 μU per ml. plasma, hepatic glucose production fell and peripheral glucose utilization fell in both young and old to the same extent. Furthermore, metabolic recycling of glucose did not differ with age. These results further strengthen the conclusion that aging primarily reduces beta cell sensitivity to glucose levels.

A computer analysis of the kinetics of glucose and insulin distribution in glucose clamp studies (maintenance of blood glucose and insulin blood levels at pre-determined values) has led to the development of a 3-compartment insulin model with preliminary evidence that the insulin content of the third pool controls the rate of glucose metabolism.

A new labeled substrate has been synthesized which greatly improves the double isotope technique developed for the determination of renin in plasma.

Longitudinal volunteers retested in the Laboratory of Behavioral Sciences six to eight years after initial testing, when they were over 60 years of age, have shown a significant decline in problem solving ability. Men under age 60 at first testing did not exhibit this effect. This decline in problem solving ability among older subjects probably results from their inability to make logical inferences from information that is already available.

Continuing studies of five patients with atrial fibrillation suggest that such patients can consistently speed and slow their hearts voluntarily. This finding may help change the idea that the ventricular rate in atrial fibrillation is a random event. Perhaps more significant is that these early results indicate that modification of some functions of the atrio-ventricular node is possible through volitional action of the patient.

Experiments with monkeys to assess the relationship between state of arousal and direction of control of pulse rate continue. While the animals are learning to control heart rate, or efficiently doing so, recordings are made of the percentage of time that high voltage, synchronized brain waves

are being produced. Previously, such activity has been linked with a quiet, awake, relaxed state. The preliminary data suggest that voluntary cardiac speeding is associated with a more "aroused" brain. Differences in brain wave activity occur without any apparent differences in motor activity.

Studies with two inbred strains of mice ($C_{57}BL/6J$, long-lived, and A J, short-lived) and F1 hybrids showed that: (a) longevity was overdominant, i.e., F1 animals lived longer than either parental strain; (b) both long-lived groups maintained a high level of activity throughout most of their life spans, whereas the short-lived animals declined in vigor disproportionately early in life; (c) in all three groups, animals which were fed isocaloric, low protein diets (4% caseine) early in life had significantly higher drive levels at maturity than did normally fed, control animals.

In collaboration with the Intermediary Metabolism Section, Laboratory of Biomedical Sciences, investigators in the Laboratory of Behavioral Sciences found that infant rats, receiving p-chlorophenylalanine and phenylalanine injections, exhibited maze learning deficits not seen in saline injected animals. The experimentally injected animals also tended to be more active and less emotional than the controls.

Program Development

Program of the GRC should be oriented and expanded in the following directions:

1. The role of immunologic responses and competence in aging.

2. The mechanisms of DNA breakdown and repair.

3. Age differences in effectiveness of control mechanisms at the cellular and organ system levels.

4. The mechanism of genetic control of life span and the modifications in life span that can be induced by environmental factors.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Gerontology Research Center
Laboratory of Molecular Aging

The Laboratory of Molecular Aging has investigated biochemical systems that control and regulate cellular processes. These studies range from the physico-chemical interaction of molecular species that determine gene expression to the bio-medical integration and coordination of entire enzymatic sequences that maintain structure and function. This increased knowledge and development of new concepts provide the scientific basis for and, thus, are fundamental to the understanding of the aging process. Representative projects selected from the research carried out in the Sections on Molecular Chemistry, Biophysics, and Intermediary Metabolism are briefly summarized, as follows.

Polymers of nucleotides and their analogs are being designed, synthesized, and evaluated for their selective uptake into cells with the view of conferring viral protection and potentially of influencing the aging process. It has been found that changes in the potent interferon inducer, poly I · poly C, that modify the polymer backbone substantially, such as loops on 2' → 5' phosphodiester bonds, lead to decreased antiviral activity. However, the complex of polyinosinic acid and poly(1-vinylcytosine) shows high interferon induction activity. It is postulated that the high antiviral activity is related to the reduction of the charge/mass ratio and to the existence of this complex in an aggregated state. These factors generally enhance their transport into cells.

Nuclear magnetic resonance, optical rotatory dispersion and spectrophotometric measurements have been used successfully to examine the binding of metal ions to ribonucleotides and deoxyribonucleotides. In a model system, the unique binding of copper(II) ions to 2' and 3' hydroxyl groups of ribonucleotides as well as to the N in the purine ring of inosine illustrate how metals can function in differentiating between and orienting nucleotides. The attachment of metal-nucleotide complexes to polypeptide templates has been employed as a probe for the study of the interaction between proteins and nucleic acids.

Studies on the interrelationships of elastolytic enzymes, antielastoproteinase factors, and elastin in the process of aging and diseases of elastic tissues have demonstrated that the elastin fibers in lungs and blood vessels of rats change qualitatively with age. The lamellar and interlamellar elastin fibers in the aorta of rats, 12 to 36 months of age, are more susceptible to hydrolysis by purified porcine pancreatic elastoproteinase than are the fibers from animals less than 1 year in age. Elastic layers around the alveoli and small blood vessels in lungs of old rats are also more readily digested. On the other hand, elastic fibers of the aorta become more resistant to hydrolysis by elastomucases as the rat ages from 1 to 24 months. Correlated examination of biomechanical properties of aortal rings indicate that after the rings are treated with the elastolytic enzymes their strength, as measured by time to break under a constant load, is increased as a function of age. Other studies revealed that elastoproteinase activity is negligible in rat serum as well as in platelets. Activity is found in the polymorpholeukocytes. Serum contains a potent inhibitor of elastoproteinase which is separable from the trypsin inhibitor.

The healing surgical wound has been used as a model system to examine the mechanisms of aging of soft connective tissues. Young animals, rats of 8 months of age, produce a significantly greater mass of scar tissue in response to incision than do 18 month old rats and this is reflected in an increased breaking strength for the scar tissue of the younger animal. The tissue produced by the younger animal is more compliant (extension at break is greater) than is that produced by the older animals. This indicates the presence of cellular mechanisms in the older animals which induce its regenerated tissues to have one of the characteristics associated with aging in connective tissue, namely — increased stiffness.

In investigations of the mechanisms of the decreases in muscular work performance during aging using the flight of insects as the model system, it was found that isolated flight muscle mitochondria from senescent flies have a significantly lower State 3 rate of respiration (the maximal rate of O_2 uptake obtainable in the presence of excess substrate, ADP, and oxygen) than those from mature flies. Neither the "resting" State 4 rate nor the ADP/O ratio is perturbed by age. The decreased State 3 rate is obtained with the two physiological substrates of this muscle, α-glycerophosphate and pyruvate + proline, indicating that the decrement in oxidative capacity is localized in the respiratory chain. Correlated ultrastructural studies reveal an unique degradation in the mitochondrial cristae in old flies. In related studies of the mechanisms of regulation at the critical enzymatic sites of carbohydrate utilization in this muscle, a novel control of phosphorylase b kinase by calcium ions and inorganic phosphate has been discovered and partially characterized. Activation of the kinase is essential for the initiation of glycogenolysis. The enzyme trehalase, which is activated when the utilization of blood sugar is initiated, has been localized in the inner membrane of the mitochondrion. An isozyme is also found in the cytosol fraction of the muscle.

For the first time, the kinetics of binding of glucose to isolated renal brush borders have been examined as part of a study on the mechanisms of active transport. At least two types of binding sites have been identified that differ greatly in their affinity for glucose. The high affinity system is half-saturated at about 0.6 μM whereas the low affinity site is half-saturated at 25 mM. Binding is readily reversible. Phlorizin competitively inhibits the high affinity binding but inhibits the low affinity system non-competitively. These and other results are consistent with the concept that binding of glucose to the brush border membrane is an integral step in the transtubular transport of the sugar.

Serial No. HD-GMA1
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Interaction of Metal Ions with Polynucleotides and Related Compounds

Previous Serial Number: Same

Principal Investigators: N. Berger(100% time)
G. Eichhorn (25% time)
J. Pitha (25% time)
Y. Shin (60% time)
E. Tarien (50% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	3.10
Professional:	2.60
Others:	.50

Project Description:

Objectives: (1) To correlate structure and reactions of metal complexes of nucleotides and polynucleotides. (2) To find specific reactions of the individual nucleotides. (3) To understand the biological significance of metal ions in nucleic acid function.

Methods Employed: The interaction of metal ions with polynucleotides and their monomeric components is studied using a variety of physical chemical techniques, e.g., optical rotatory dispersion, nuclear magnetic resonance, ultraviolet and infrared spectrophotometry. From these data the site of interaction of the metal can be correlated with its effect on the reactions of the nucleic acids.

Major Findings: A. Nuclear magnetic resonance studies of the binding sites of Cu(II) ions to purines in nucleosides and nucleotides. It had previously been demonstrated that Cu(II) ions bind to multiple sites on the adenine ring, and that the binding site varies with the position of the phosphate group in a nucleotide. It has now been established that the paramagnetic broadening effect used to characterize the binding sites is precise in pinpointing the binding sites. In tubercidin, an analog of

adenosine in which the N-7 binding site is replaced by carbon, Cu(II) ions do not produce broadening of the H-8 peak that is broadened in adenosine; however, H-2, which is adjacent to potential binding sites N-1 and N-3, is broadened. Thus, the broadening effect due to binding of a paramagnetic ion is not transmitted from one ring of the purine to the other. The paramagnetic broadening effect was then used to determine the nature of the Cu(II) binding sites in IMP and in inosine. Both H-8 and H-2 resonances are broadened in the nucleotide as well as the nucleoside, demonstrating metal binding to N-7 and the N-1 region. When the N-1 site of inosine is blocked by a methyl group, binding occurs at N-7 only, and when N-7 is methylated, binding occurs only in the N-1 region. In inosine N-7 is preferentially bound at high pH and N-1 is preferred at low pH; at intermediate pH both sites are bound. Heating also results in binding to N-1. Removal of a proton from N-1 generally makes it suitable for metal binding.

B. Binding of Cu(II) to ribose hydroxyls in nucleosides and the difference in binding to ribosides and deoxyribosides. Binding of Cu(II) to the ribose hydroxyl groups is demonstrated in DMSO by broadening of NMR peaks of ribose by Cu(II) acetate. The interaction of Cu(II) acetate in DMSO with inosine, 1-CH_3-inosine, and 7-methyl-inosine is accompanied by a marked decrease in absorbance of the visible peak of Cu(II) acetate and a marked increase in the near u.v. absorption. In contrast deoxyinosine produced no increase in the near u.v. and a slight increase in the visible. Studies with a large series of nucleosides and deoxynucleosides revealed that all of the former, regardless of the nature of the base, produced a similar hypochromicity ($>50\%$) in the visible bands and a marked increase ($>20x$) in the near u.v. band of Cu(II) acetate. These effects were not produced by any of the 2'-deoxynucleosides, by nucleosides with hydroxyls blocked by acetate, by 3'-deoxyadenosine, or by cytosine arabinoside. Thus the presence of both free 2' and 3' ribose hydroxyls in a cis position is required to produce this complex. Continuous variation studies with inosine and its analogs demonstrated the formation of 2:1 Cu(II) nucleoside complexes. These complexes are not formed by $Cu(NO_3)_2$ until acetate ion is added. Since Cu(II) acetate exists as a dimer with four acetate molecules bridging two copper atoms joined by a metal-metal bond, it appears that the 2' and 3' hydroxyl groups of the ribose coordinate to these copper atoms by replacing one of the acetate groups.

In the formation of this complex metal ions specifically recognize and bind ribonucleosides as distinguished from deoxynucleosides. This is of paramount importance since both DNA and RNA polymerase employ divalent metal ions to identify and bind nucleotides or deoxynucleotides. The 2:1 Cu(II) nucleoside complex has features that can serve as a model for the metal-bound polymerase enzymes. In the model ribonucleosides are recognized by two Cu(II) atoms which are maintained in proper conformation by bridging acetate molecules. It is possible that the polymerase enzymes employ metal ions in an analogous fashion to orient the nucleosides.

C. Nuclear magnetic resonance studies on the binding of Mn(II) to adenosine and AMP. Because of the biological importance of Mn(II) ion in nucleic acid reactions, e.g. the polymerase reactions mentioned above, some studies have been conducted on the broadening of the resonances of adenosine and AMP by

Mn(II), demonstrating that the NMR mainly reflects binding to the phosphate group. Base binding is also detected, and N-7 is preferentially broadened regardless of the position of the phosphate. Thus Mn(II) binding appears to reflect more directly the relative binding tendencies of the base sites, and is not so much influenced, as is Cu(II), by other forces in the molecule.

D. NMR studies of the unwinding of poly I•poly C in the presence or absence of Cu(II). The unwinding of the poly I•poly C double helix has been investigated by NMR. The peaks that are broadened at low temperature (4°) emerge when the temperature is increased and the strands separate, so that the base peaks of each polymer can be clearly identified. In the presence of added Na^+ ions the helix is stabilized and characteristic peaks emerge at higher temperatures.

When the poly I•poly C complex is melted in the presence of Cu(II) ions the base peaks emerge from the double helix with the poly I, H-8 and the poly C, H-5 peaks specifically broadened. The broadening effects of Cu(II) persist even in the presence of excess Na ions which might be expected to displace metal ions from phosphate. This indicates that Cu(II) is bound to the bases at N-7 on poly I and N-3 on poly C and that the association between metal and polynucleotides persists at temperatures as high as 80°C where unwinding is complete. The demonstration that Cu(II) ions are coordinated to bases in poly I•poly C, with the similar demonstration in poly A•poly U accounts for the ability of these metal ions to weaken base paired structures and unwind the double helix.

In contrast to poly A•poly U where Cu(II) ions were found to preferentially associate with the poly A components, Cu(II) ions are coordinated to both strands of the poly I•poly C complex. Since the metal ions remain coordinated to the bases of opposite strands, at high temperatures, it is apparent that in the proper location, a metal ion could be coordinated between bases on opposite strands which would maintain bases in register and facilitate rewinding, when favorable conditions return.

E. The control of polynucleotide conformation by metal ions. It was previously demonstrated by optical rotatory dispersion that different metal ions have quite different effects upon the conformation of polynucleotides. We have now found that the effect of metal ions upon the polynucleotide conformation can be fine-tuned by other conditions such as ionic strength, so that any conformation can be stabilized with the aid of the suitable metal ion. Thus Ni^{2+} or Co^{2+} can stabilize the random coil form of poly A at pH 6 at 1×10^{-4}M salt concentration, whereas the single helix is stabilized by the same metal ions above 1×10^{-3}M salt.

Multistranded polynucleotides are stabilized through phosphate binding when the strands are not held together by H^+ ions (poly G, poly I, poly U), but they are destabilized when H^+ ions are so involved. Ordered structures are generally destabilized by base binding.

Mg^{2+} ions and Cd^{2+} ions are least subject to control because the former generally affects conformation through phosphate binding while the latter operate through base binding. Ni(II), Co(II), Mn(II) and Zn(II) are more

susceptible to solution conditions, since they act by binding to both base and phosphate. Thus, Ni^{2+} readily produce a random coil from poly A single helix and poly G double helix because the phosphate binding ability cannot further stabilize these structures, since the phosphate groups are far apart and charge neutralization cannot increase the helical content; hence Ni(II) ions binding bases produce an unwinding effect. On the other hand, when helices are capable of further stabilization by metal binding, because the phosphate groups are crowded and repel each other, Ni^{2+} ions are very effective in helix stabilization, e.g. poly U and poly I. Zn(II) ions frequently produce results similar to those of Ni(II) ions, but they bind the bases more strongly, and therefore tend more readily to disorganize ordered structures. Thus the T_m of poly I is lowered almost as much by Zn(II) as by Cd(II).

In general, purine polynucleotides are more readily converted to the random coil configuration than pyrimidine polynucleotides. The poly A single helix can be converted to a random coil by all transition metals, but not poly C. The poly I triple helix is partly destabilized by the same metal ions (Ni(II), Co(II), Zn(II)) that stabilize the poly U double helix.

F. Infrared spectroscopy. In the previous year a new method was developed for the study of the interaction between metals and polynucleotides, consisting of the in situ modification of DNA films. Presently the method has been applied to calf thymus DNA and the following cations: H^+, Li^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+}. With Li^+, Mg^{2+}, Ca^{2+} and Mn^{2+} the main phenomenon observed is cation exchange and binding, with H^+ and Cu^{2+} the DNA strands separate. Thus the ions which are good polarizing agents bring about the unwinding of the DNA under these conditions, which in some respects resemble those in the cell more than those in aqueous solution. Metal ions which do not readily polarize do not produce any changes in DNA conformation.

Conformational equilibria in nucleosides and their derivatives have considerable importance for the study of enzyme specificities and their influence on metal ions. There are two basic conformers: syn and anti. In the majority of crystal structures which have been analyzed the latter conformation is preferred and also several enzymes are known which use only the anti conformer. If a compound cannot attain the anti geometry it is not used as a substrate. The stability of syn and anti forms can be changed by coordination of the nucleoside to a metal and if the energy barrier between the two forms is low enough, a complete switch from one conformation to the other can be achieved, with subsequent blocking or preference of some conformation-sensitive enzymatic pathway. To lay the foundation for such studies, the energy barrier between conformations was studied in simple substituted derivatives of uridine and adenosine without the presence of metal. A method was worked out which enables the direct detection of both syn and anti conformations. It has been found that the relative stability of the syn and anti forms depends on the hydrogen bonding possible in the respective derivatives and that uridine and adenine derivatives exist in both syn and anti conformations. The energy differences between conformers must be comparable to that of hydrogen bonding (2-4 kcal) and so should be capable of manipulation by coordination of a metal to a nucleoside.

Significance to Bio-medical Research and the Program of the Institute: Localization of metal binding sites on nucleotides and nucleosides demonstrates some of the ways in which metal ions can interact with these compounds in biochemical reactions. Of particular interest is the demonstration of a system in which metal ions can identify and bind to nucleosides as distinct from deoxynucleosides. Study of this model complex provides an understanding of the mechanism of this recognition process by the polymerase enzymes. Identification of the binding sites in the polymers contributes to the understanding of the mechanism of alteration and preservation of nucleic acid structure.

The ability of metal ions to control the conformation of polynucleotides signifies that metal ions in biological systems can determine whether or not the polynucleotide is in a favorable conformation for its genetic activity.

Proposed Course of Project: Preliminary studies have been initiated to determine binding sites of other metal ions on nucleic acids and components with some similarities and differences being observed. These will be explored in terms of their effect on nucleic acid structure. Conditions are also being developed so that DNA and other natural nucleic acids can be examined by NMR.

The characteristics of the metal-ribose model will continue to be investigated with an attempt to relate this to the recognition process by the enzyme.

Honors and Awards:

1. Gunther Eichhorn was invited to present a lecture on "The Interaction of Metals with Nucleic Acids at a Symposium on Advances in Biopolymers at the Middle Atlantic Regional Meeting of the American Chemical Society, Baltimore, February, 1971.

2. Gunther Eichhorn was invited to present a lecture on "The Interaction of Metal Ions with Purine Nucleosides and Nucleotides" at the Symposium on The Purines: Theory and Experiment, Jerusalem, Israel, April, 1971.

3. Gunther Eichhorn was invited to present a lecture at a Symposium on Bioinorganic Chemistry at the Central Regional Meeting, American Chemical Society, Cincinnati, Ohio, June, 1971.

Publications:

Berger, N. A., and Eichhorn, G. L., "The Interaction of Metal Ions with Polynucleotides and Related Compounds. XIV. NMR Studies of the Binding of Cu(II) to Adenine Nucleotides." Biochemistry, in press.

Berger, N. A., and Eichhorn, G. L., "The Interaction of Metal Ions with Polynucleotides and Related Compounds. XV. NMR Studies of the Binding of Cu(II) to Nucleotides and Polynucleotides." Biochemistry, in press.

Eichhorn, G. L., Berger, N. A., Butzow, J. J., Clark, P., Rifkind, J., Shin, Y. A., and Tarien, E., "The Effect of Metal Ions on the Structure of Nucleic Acids". In Gould, R. F. (Ed.): Bioinorganic Chemistry. Advances in Chemistry Series 100, pp. 135-154, (1971).

Pitha, J., "In Situ Modification of Polynucleotide Films for Infrared Study". Biochim. Biophys. Acta, 232, 607 (1971).

Pitha, J., "Hydrogen Bonding in Derivatives of Adenosine and Uridine". Biochemistry 9, 3678 (1970).

Serial No. HD-GMA2
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Degradation of Nucleic Acids by Metal Ions

Previous Serial Number: Same

Principal Investigators: J. Butzow (100% time)
G. Eichhorn (20% time)
E. Tarien (50% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	1.70
Professional:	1.70
Others:	0.00

Project Description:

Objectives: (1) To render metal ion degradation of polynucleotides useful in the detection of nucleotide sequence and in selective degradation of ribonucleic acids. (2) To study the mechanism of the metal degradation reaction.

Methods Employed: Details of the influence of nucleotide sequence in ribonucleic acids on the zinc cleavage reaction and of the mechanism of the reaction are followed by means of kinetic measurements of degradation and determination of the degradation products, using dimers and trimers of known primary structure as substrates.

The weak zinc ion depolymerization of DNA is investigated by measuring the production of the very first few breaks in its individual strands.

Major Findings: A. Nucleotide specificity in the zinc(II) cleavage of polyribo- and oligoribonucleotides. We have previously demonstrated that a high degree of nucleotide specificity can be obtained in the zinc(II) cleavage of RNA. We have now made detailed analyses of rate studies of the zinc degradation at neutral pH of several triribonucleoside diphosphates. In each case, the phosphodiester bond nearer the 5' end of the chain, that is, the bond linking a nucleoside which bears a 3' phosphate, is the more susceptible to cleavage. (We have shown that the existence of

this preference is related to an effect of such a 3' phosphate;(see sect. B). The degree of the cleavage preference is affected by the nucleotide sequence: it is somewhat greater for ApApA than for ApApU, much greater for ApApG than for ApApU, and much greater for UpUpG than for UpUpU, reflecting the greater tendency to cleave next to U and the lesser tendency to cleave next to G which we have observed in the zinc degradation of RNA. Moreover, the presence of G at the 3' end leads to a marked increase in the actual cleavage rate of the phosphodiester bond nearer the 5' end. The sequence-specific differences in the zinc cleavage of these trimers might be attributed to differential base stacking—no base pairing is expected to occur in the particular trimers. Since, although we find that a small degree of base stacking does remain under the reaction conditions for ApApA, ApApG, and ApApU, we find none for UpUpU and UpUpG, we are led to consider mediating influences in addition to base stacking (and base pairing). The binding of transition metal ions to the bases represented here is known to be greatest for guanine and poorest for uracil; thus, our findings offer evidence that base binding of zinc ion could play a critical role in determining the cleavage pattern in an oligo- or polyribonucleotide.

B. Effect of the adjacent phosphate group on zinc(II) cleavage of a phosphodiester bond in a ribonucleic acid. We have observed that the (3'-5') phosphodiester bond nearer the 5' end of a triribonucleoside diphosphate is the more susceptible to zinc(II) cleavage (at neutral pH). Prompted by preliminary evidence that a mixture of ApA2'p plus ApA3'p is much more readily degraded by zinc than ApA, we conducted rate studies of the zinc degradation of adenylate dimers possessing a 3' end phosphate group in the three possible configurations which can occur in the course of zinc degradation of polyriboadenylate: for ApA2'p, for ApA3'p, and for ApA2'-3'(cyclic)p. The rates of internal cleavage of ApA, ApApA, and these adenylate dimers lie in the order: ApA3'p $\gtrsim$ Ap/ApA > ApA2'p $\gtrsim$ ApA2'-3'(cyclic)p $\gtrsim$ ApAp/A $\gtrsim$ ApA. Thus, the rate of zinc cleavage of an internal phosphodiester bond is influenced by the existence, configuration, and charge of a neighboring phosphate group (under the reaction conditions of neutral pH and small excess zinc ion). Charge repulsion between the phosphodiester and the adjacent phosphates apparently is involved.

The effect of the 3'phosphate group of the nucleotide moiety linked by the phosphodiester bond being cleaved makes understandable several phenomena which we have encountered in our studies of the zinc degradation reaction. Besides rationalizing the cleavage preference in a trinucleoside diphosphate and the variation of cleavage susceptibility with chain length, the action of an adjacent phosphate group (together with the fact of intermediate production of the 2'-3' cyclic phosphate) essentially can explain the order of appearance of all the fragments found in the course of zinc degradation of a homopolymer.

C. Difference between DNA and RNA in susceptibility to zinc(II) cleavage. We have extended our measurements of breaks occurring at neutral pH in the presence and in the absence of zinc(II) ion, in the individual strands of calf thymus DNA and polyriboadenylate, to temperatures below (50°) and above (80°) the transition of the DNA from its native double stranded to its single stranded conformation. Breakage in the individual strands—sensitively

determined by means of changes in sedimentation coefficient under conditions of strand separation—is detected for the DNA after exposure in either conformation. The cleavage rate of the DNA, however, is very small compared to that of the polyriboadenylate (entirely single stranded), in the presence of zinc: as much as 4000-fold less when the DNA is double stranded and about 1000-fold less when single stranded.

Significance to Bio-medical Research and the Program of the Institute: The ability of metal ions to degrade ribonucleic acids is of interest in terms of a model for the mechanism of action of nucleases. The degradation reaction could account for deleterious effects on RNA through accumulation of metal ions during growth, development and aging of organisms. Utilization of the reaction can be of importance in detection of nucleotide sequence in biologically functional ribonucleic acids. The great difference in susceptibility to metal ion cleavage between DNA and RNA represents a fundamental difference between these two classes of nucleic acids, with important analytical applications, such as selective elimination of RNA from complex biological systems.

Proposed Course of Project: In view of the nucleotide specificity which we have so far demonstrated for the zinc cleavage reaction, we shall undertake a detailed study of metal ion cleavage of 5S ribosomal RNA, as a polynucleotide of unique sequence. We shall place additional emphasis on metal ion modification of polymer structure and the influence of polymer structure on the cleavage reaction—through study of the degradation and of the physical properties of both 5S RNA and various ordered and partially ordered polynucleotide complexes to which metal ions are bound. In order to assess in more detail the influence of the pentose on metal ion cleavage of polynucleotides, we plan to study the degradation of deoxyhomopolymers and complexes of deoxyhomopolymers with ribohomopolymers.

Honors and Awards: None

Publications:

Eichhorn, G. L., Tarien, E., and Butzow, J. J., "Specific Cleavage Effects in the Depolymerization of RNA by Zinc(II) Ions". Biochemistry, in press.

Butzow, J. J., and Eichhorn, G. L., "On the Mechanism of Degradation of Polyribonucleotides and Oligoribonucleotides by Zinc(II) Ions". Biochemistry, in press.

Serial No. HD-AG30
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Function of Metal Ions in Enzymatic Reactions

Previous Serial Number: Same

Principal Investigators: P. Clark (100% time)
G. Eichhorn (30% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	1.30
Professional:	1.30
Others:	0.00

Project Description:

Objectives: (1) To convert non-specific nuclease enzymes into base-specific cleavage reagents useful in the determination of nucleotide sequence. (2) To elucidate the mechanism by which metal ions activate or inhibit enzymatic reactions.

Methods Employed: DNAse is an enzyme that cleaves the internucleotide bonds of DNA rather non-specifically. Since the enzyme presumably recognizes a nucleotide unit in the DNA structure, the reaction of any nucleotide base with a metal ion should prevent reaction of the enzyme with that nucleotide, so that preference would be exhibited for nucleotide sites that do bind the metals. Since different metal ions react preferentially with different sites on the DNA, it is expected that participation of a variety of metal ions in the DNAse reactions will block cleavage at different locations, thus changing the specificity of the enzyme in a variety of ways. The metal-DNA complexes are reacted with the enzyme, the resulting oligonucleotides are treated with phosphatase to remove terminal phosphate, and then with venom phosphodiesterase to hydrolyze to nucleotides and nucleosides. Analysis of the nucleosides indicates points of cleavage of DNA by DNAse.

The mechanism of activation or inhibition of the enzymes by metal ions is determined by kinetic studies of enzymatic reactions, comparing the effect of various metal ions on the reactions. Physical chemical studies are

carried out to determine the characteristics of the binding of the metal to the enzyme that are responsible for the biological functions.

Major Findings: A. The binding of Cu(II) and DNA to DNAse. It has been previously shown that Cu(II) ions have a profound effect on the specificity of the DNAse reaction. In order to gain insight into the nature of the interaction of Cu(II) with DNAse, the optical spectra and ORD curves of solutions of Cu(II) and DNAse have been further explained. By analogy with studies on other proteins, it appears that the Cu(II) binds to an amino nitrogen, a peptide nitrogen and a hydroxyl group of tyrosine.

The ORD curve of DNAse is modified by the addition of 2 moles DNA per mole of protein in that the 229nm minimum is shifted to a broad range between 230 and 24 nm. The contribution of the DNA is wiped out by that of DNAse. These findings suggest that the structure of DNA is completely deformed by the interaction which also causes a slight perturbation on the enzyme molecule.

B. Inhibition of DNAse hydrolysis by Ag(I) and Hg(II). Because of the successful application of the reaction of Cu(II) with DNAse, to produce a more specific reaction, studies have been carried out to determine whether Ag(I) and Hg(II) would promote a unique cleavage specificity. Ag(I) and Hg(II) alone do not activate the DNAse I hydrolysis of DNA. However, if either silver or mercury is present to the extent of one atom of metal ion to five DNA nucleotides, this complex can be hydrolyzed by DNAse in the presence of Mg(II). This ratio of metal ion to DNA bases is the only one which is useful in the hydrolysis of DNA by the enzyme. The reaction is very slow, usually requiring three hours to break three to five per cent of the phosphodiester bonds. The average chain length of the perchloric acid soluble fraction from the mercury hydrolysis is 12 to 16 nucleotides, whereas with Mg alone the average chain length of acid soluble material is 5 to 6 nucleotides. Preliminary results on the analysis of nucleosides at the cleavage site show low amounts of guanosine and adenosine and mostly pyrimidines present, suggesting that the main cleavage of the phosphodiester bond occurs adjacent to a pyrimidine base, producing a 5' pyrimidine nucleotide end group. The preliminary results on the hydrolysis of the silver-DNA complex indicate a large amount of adenosine and small amounts of cytidine and guanosine.

C. Book on Inorganic Biochemistry. 32 of the 34 chapters of this book have been received. Work is proceeding on the editing of the chapters.

Significance to Bio-medical Research and the Program of the Institute: The overall project derived its importance from the application of the principles of inorganic chemistry to biological problems. The publication of a book on this subject will contribute immeasurably to progress in this field. The utilization of the metal inhibited deoxyribonuclease reaction in nucleotide sequence determination can lead to an understanding of the message in DNA, which contains the genetically determined information for growth, development and aging.

Proposed Course of Project: It is hoped that the book on Inorganic Biochemistry will be completed this year. We hope to continue the study of the effect of Hg(II) and Ag(I) on the specificity of DNAse.

Honors and Awards: NONE Publications: NONE

Serial No. HD-GMA3
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Structure of Nucleoproteins

Previous Serial Number: Same

Principal Investigators: G. Eichhorn (20% time)
J. Rifkind (50% time)
Y. Shin (40% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	1.60
Professional:	1.10
Others:	.50

Project Description:

Objectives: (1) To determine whether specific interactions occur between nucleotides and polypeptides and between DNA and polypeptides. (2) To determine the effect of metal ions on these interactions.

Methods Employed: The interactions between nucleotides and amino acids in nucleoproteins may be responsible for specificities in nucleoprotein binding that could be important in cellular differentiation and aging. Optical rotatory dispersion techniques, which are sensitive to changes in the conformation of molecules, are used to determine the changes produced by the interaction of polypeptides, nucleotides and metals. Similar methods are applied to the study of polypeptide-DNA interaction and supplemented by melting curves and titration curves. The data are analyzed to deduce the structural changes produced by these interactions.

Major Findings: A. Reversibility in conformational changes in DNA-polylysine interaction. It has been previously shown that metal ions enhance the deformation to which the enveloping polypeptide subjects the DNA double helix. Metal ions can thus increase the ability of the polypeptide to "protect" the DNA. We have now discovered that, in the process of heating the DNA-polylysine supercoil stabilized by metal ions in the presence of excess DNA, the ORD curve is transformed from that characteristic of the supercoil to that of the Watson-Crick double helix. Thus excess DNA is

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capable of competing with the "protected" DNA for its polylysine cover. This finding attains significance as a result of the discovery by Felsenfeld that chromatin contains substantial portions of DNA that is unbound to protein. The free DNA can presumably compete with bound DNA for the protection protein.

B. The reaction of zinc-AMP complexes with polypeptides. It has been previously shown that a very specific zinc-AMP complex, characterized by a very high optical rotatory strength, is produced in the presence of a number of different polypeptides. The metal must be zinc and the nucleotide must be 3' or 5' AMP. We have now shown that this complex will form not only with conventional polypeptides but also with the followsing substances: 1) polyethylene imine, a globular polyamine containing a mixture of primary, secondary and tertiary amines, 2) DEAE-dextran, a positively charged polysaccharide, and 3) a neutral Dowex fraction. This evidence confirms the non-specificity of the polymer requirement in contrast to that of metal and nucleotide. Apparently the polymer serves as a template on which the zinc-AMP becomes attached.

The nature of the protein attracting zinc complex has now been considerably clarified. When the three phosphate isomers of AMP (2',3' and 5') are titrated in the presence of zinc, the titration curves of 3' and 5' AMP are found to be very similar to each other and different from the 2'AMP curve. Specifically, the zinc(II) 2'AMP complex reacts with 0.5 mole of base, comes to equilibrium and then reacts with 1 mole of base before reaching a second equilibrium point. This behavior indicates that two zinc ions complexed to 2'AMP are held together by a hydroxyl bridge. In contrast, the titration of Zn(II) 3'AMP and Zn(II) 5'AMP proceeds by simultaneous utilization of 2 moles of base at a pH much higher than that at which zinc(II) alone is hydrolyzed.

The titration results thus show that the same AMP isomers (3' and 5') that produce the unusual ORD effect with Zn(II) and polypeptides or other polymers have similar titration behavior with zinc(II), and differ from 2'AMP, which does not produce the ORD characterized complex. Circular dichroism studies reveal the presence in the 3' and 5' complexes of two bands of similar magnitude but opposite sign in the region of the uv absorption band. This is an indication of parallel stacking. The two bands are in the same position but inverted in the two isomers, indicating that they are mirror images. The rotatory strength of the 5' complex is of similar magnitude to poly A, while for the 3' complex it is an order of magnitude greater. The complexes are very stable to heat; the ORD spectrum of the Zn(II) 5'AMP complex begins to diminish in intensity at 75° but even at 98° the rotation has decreased by only 25%. The 3' complex is somewhat less stable, losing 80% of the rotation due to the complex at 98°.

The specific requirement of adenine in the 3'nucleoside is just as previously established for the 5' case. The substitution of 3'GMP produces no effect, and 3'deoxyAMP produces a greatly diminished effect. With 3'AMP also zinc(II) is the only metal ion that works. Titration curves of Zn(II) with 5'AMP and 5'dAMP are identical, indicating that the zinc binds to the same site in both nucleotides. The difference in the ability to form stacked complexes

thus lies in the different sugar conformation of ribo- and deoxyribonucleotides.

A clue to the binding site of zinc(II) on the adenine ring in these complexes comes from the fact that substitution of a methyl at the N-1 position produces no complex. Methyl substitution at the amino group does produce a complex, but without parallel stacking. It thus appears that the zinc binds to the N-1 site on the adenines as well as to the phosphate group. The possibility that the binding occurs at N-7, but is prevented by conformational changes through substitution at N-1, cannot be excluded.

To study the possibility that positively charged amino acids in histones could form complexes with Zn(II) AMP similar to those produced with polylysine, the reaction of Zn(II), 3' or 5'AMP and a copolymer containing 42% glutamic acid, 28% lysine and 30% alanine was studied. The failure to form a complex with this polymer could be attributed to the randomness of the amino acid sequence, thus providing no regularity as a template, or to the rigidity of the polymer which exists in helical form as a result of the mutual charge neutralization of the lys and glu residues.

Significance to Bio-medical Research and the Program of the Institute: The binding of a metal nucleotide complex to polypeptides and other polymers, with high specificity for metal and nucleotide and low specificity for the nature of the polymer, suggests that preformed metal nucleotide complexes can react with enzymes such as DNA and RNA polymerase. The finding that unbound DNA competes with DNA ligated to polylysine for binding sites on the polylysine suggests that protein can move about on an incompletely covered DNA molecule; such a possibility is not favorable for the hypothesis that histones function to block specific portions of the genetic code.

Proposed Course of Project: We intend to study the specific binding sites of protein to nucleic acid using nuclear magnetic resonance techniques.

Honors and Awards: None

Publications:

von Hahn, H. P., Heim, J. M., and Eichhorn, G. L., "The Effect of Divalent Ions on the Isolation of Proteins from Rat Liver Nucleoprotein". Biochim. Biophys. Acta 214, 509 (1970).

Serial No. HD-GMA4
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Age Dependent Alterations of Genetic Information

Previous Serial Number: Same

Principal Investigators: N. Berger
G. Eichhorn

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Project Description: The work in the specific area studied was discontinued as H. V. Samis announced getting some results in a similarly designed project. Comparison of Dr. Samis's results and ours shows that continuing in precisely the same line of approach in order to give broader confirmation and supplement the already known observations is not justified.

Publications: None

Serial No. HD-GMA5
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Analogs of Nucleic Acids

Previous Serial Number: Same

Principal Investigator: J. Pitha (70% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	0.70
Professional:	0.70
Others:	0.00

Project Description:

Objectives: (1) To study polymers which have the heterocyclic moieties of nucleic acids, but differ in connecting backbone. (2) By comparison of the synthetic with the natural molecules to isolate the structural features of the latter that are of biochemical significance. (3) To prepare polymers consisting of biologically active units which will be designed in such a way that they will be taken up by mammalian cells and then, inside, will disintegrate.

Methods Employed: Polymeric analogs are prepared by radical polymerization or condensation reaction from suitable monomers. Their association with polynucleotides are studied with a range of spectral methods. Polymeric carriers are prepared by condensation reactions and tested on fibroblast in tissue cultures.

Major Findings: A. Biophysical aspects. The mutual interactions of four neutral analogs of nucleic acids, poly(1-vinyluracil), poly(1-vinylcytosine), poly(9-vinyladenine) and poly(9-vinylhypoxanthine), and their interactions with polyribonucleotides, were investigated in dilute aqueous solutions. Poly(9-vinylhypoxanthine) shows considerably less tendency for any complexing than the other vinyl polymers. The formation of complexes follows the specificity pattern of polynucleotide interaction, except that the nature of the interactions is modified by two additional factors. First, because the bases of a vinyl polymer cannot completely pair with a single strand of any other polymer, some of the bases of the complexed vinyl polymer are

potentially free to bind to additional strands. Secondly, because of the incomplete base pairing the strand-strand interactions are weaker and could be offset by coulombic repulsion forces. As a result, the nature of an interaction is determined by the electric charges. If neither component carries any charge, which is the case when both are vinyl polymers, no coulombic repulsion acts in the system and the growth of the complex continues until a high aggregate is formed. When one component is neutral (vinyl polymer) and the other negative (polynucleotide), a soluble, non-aggregated complex is formed, the repulsive forces acting against progressive joining of more negative strands. These complexes have the following characteristics: a) lower stability than the corresponding polynucleotide-polynucleotide complex, b) broad and incompletely reversible melting, c) filamentous appearance upon electron microscopic examination (observed for the poly(1-vinyluracil)-polyadenylic acid complex. Similarly, the complex between poly(9-vinyladenine) with intercalated negative detergent and poly(1-vinyluracil) is non-aggregated. When both components carry an electric charge of the same sign, as in the case of two protonated vinyl polymers, or of a negative polynucleotide and a vinyl polymer with intercalated anionic detergent, then no complex is formed; apparently the incomplete pairing cannot overcome the coulombic repulsion between two strands.

B. Biological aspects. The following aspects of polymeric analogs have been investigated.

a) In co-work with Drs. L. E. Orgel and P. M. Pitha it was found that poly(1-vinyluracil) can act as template for polymerization of cyclic AMP.

b) In co-work with Dr. P. M. Pitha the antiviral resistance produced by polymeric analogs was elucidated. The potent interferon inducer, poly I·poly C, was used as a starting point for the different structural changes. It has been found that changes that modify the polymer backbone substantially, such as loops on 2' ⟶ 5' phosphodiester bonds, lead to decreased antiviral activity. Unexpectedly, however, the complex of polyinosinic acid and poly(1-vinylcytosine), which is a much more distantly related analog of poly I·poly C, shows high activity. It has been postulated that the high activity is related to the reduction of the charge/mass ratio and to the existence of this complex in an aggregated state; these are two factors that generally enhance the uptake of compounds by cells.

Further, I have participated in studies with the following results.

a) Poly(1-vinyluracil) and poly(9-vinyladenine) do not serve as template in protein synthesis (Dr. D. Grünberger).

b) The shape and size distribution of complexes: polymeric analogs - polynucleotides by electron microscopy (Drs. Kleinschmidt and P. M. Pitha).

Significance to Bio-medical Research and the Program of the Institute:
With one complex studied a considerable antiviral protection of mammalian cells was achieved. Comparison with other active interferon inducers enabled us to formulate a hypothesis on the critical importance of the cellular uptake for interferon induction. From the biophysical point of view the study

of polymeric analogs showed that kind of base has a much stronger influence on properties of a polymer in the case where backbone is electroneutral (vinyl polymer) than polyanionic (nucleic acid).

Proposed Course of the Project: The present results have demonstrated the importance of the proper molecular form of a compound for the amount of its cellular uptake and consequently its biological effects. This line will be followed by design and synthesis of macromolecular compounds containing hydrolyzable chelating groups or antioxydants, so that eventually it will be possible to transport large amounts of metals or antioxydants across the cell walls. Attention will be given to biological side of the project with hope to find and learn methods proper for the study, including the necessary tissue culture techniques.

Honors and Awards: None

Publications:

Pitha, J., "Tautomerism of 2-Ethoxy-4-pyrimidinone". J. Org. Chem., 35, 903 (1970).

Pitha, J., "Self-Association in Axial β-Hydroxycyclohexanones". J. Org. Chem., 35, 2411 (1970).

Pitha, P. M., and Pitha, J., "Preparation and Properties of Poly-9-vinyladenine". Biopolymers 9, 965 (1970).

Pitha, J., and Pitha, P. M., "Antiviral Resistance Produced by the Polyinosinic Acid-Poly(1-vinylcytosine) Complex". Science, in press.

Serial No. HD-AG17(8)(c)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Relation of Structure and Function in Hemoglobin

Previous Serial Number: Same

Principal Investigator: J. Rifkind (50% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	0.75
Professional:	0.50
Others:	0.25

Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin. (2) To determine the role of the protein in controlling the binding of ligands to hemoglobin. (3) To study the mechanisms for maintaining hemoglobin in its functional form.

Methods Employed: Hemoglobin is purified by gel filtration. The autoxidation, oxygenation and dissociation of hemoglobin are investigated in the presence of various low molecular weight substances. Binding studies to hemoglobin are performed with some of these substances to help to understand their mechanism of action. These studies help to elucidate the factors which control the oxygenation of hemoglobin and which maintain hemoglobin in its reduced functional form.

Major Findings: A. Glutathione prevents the autoxidation of hemoglobin. We had previously found that the reduced glutathione which is present in the erythrocyte can retard the rate of autoxidation of purified hemoglobin. We have now found that oxidized glutathione is also very effective in preventing the autoxidation of hemoglobin when higher concentrations are used. Oxidized glutathione is not a reducing agent and therefore most probably functions by binding to hemoglobin and thereby protecting the heme from being oxidized.

Disulfides, such as oxidized glutathione, are known to react with sulfhydryl groups, and the sulfhydryl groups located on the β-93 residues of

hemoglobin are known to be reactive. In order to test the hypothesis that oxidized glutathione inhibits autoxidation by reacting with the β-93 sulfhydryl groups, we investigated the effect of mercuric chloride and N-ethylmaleimide. These are both sulfhydryl reagents which are structurally completely unrelated to oxidized glutathione. They also inhibit the autoxidation of hemoglobin. While the β-93 residues are involved in the oxygenation, dissociation, and even denaturation of hemoglobin, they have not previously been implicated as playing a role in controlling the autoxidation of hemoglobin.

Reduced glutathione is a very poor reducing agent. The effective inhibition of autoxidation by reduced glutathione, therefore, must involve an additional mechanism. While reduced glutathione is not a sulfhydryl reagent, we found evidence for a relatively effective blockage of the β-93 sulfhydryl groups in the presence of reduced glutathione. We believe that this reaction involves reactive thiyl radicals which are produced whenever ferrihemoglobin is reduced by glutathione.

In the normal erythrocyte there is an enzyme system which very effectively reduces any ferrihemoglobin which is formed, and glutathione probably does not play a significant role in preventing the accumulation of ferrihemoglobin. However, there is evidence that some β-93 residues are blocked by glutathione especially in certain hemolytic anemias such as hemoglobin H disease and also in aged erythrocytes. These results suggest that under conditions of stress the reaction of glutathione with the β-93 sulfhydryl groups may play a role in helping to maintain functional hemoglobin.

B. Autoxidation can be controlled by chelating metal ions. We had previously found that ethylenediaminetetraacetic acid (EDTA) drastically reduces the rate of autoxidation. More recently we have performed careful experiments to determine whether EDTA interacts directly with hemoglobin, or whether it removes metal ions which can catalyze the oxidation of hemoglobin.

We found that one mole of EDTA binds per tetramer of hemoglobin. However, no binding is observed until after the EDTA has fully stabilized the hemoglobin. Thus, in the concentration range in which EDTA stabilizes hemoglobin it removes something from hemoglobin instead of binding to hemoglobin. We have also shown that triethylenetetramine, which has an affinity for metals similar to EDTA, also stabilizes hemoglobin even though it is structurally very different from EDTA.

The mechanism by which metals catalyze the autoxidation seems to involve the binding of a metal which can participate in the transfer of electrons from the Fe(II).

We have studied the binding of Zn(II) to hemoglobin and find that approximately one Zn(II) per subunit is bound very strongly. We also found that of the divalent metal ions Cu(II) is by far the most effective catalyst for the oxidation of hemoglobin. In fact, low concentrations of many metal ions do not measurably enhance the autoxidation. This behavior seems to be related to the fact that Cu(II) can readily be reduced to Cu(I).

Trace metals are present in the erythrocyte and in fact copper is thought to be essential for erythropoiesis. However, in the functional erythrocyte these metals are complexed with various components of the cell. For example most of the copper is bound to erythrocuprein. Our results on the autoxidation of hemoglobin suggest that when the erythrocyte is disrupted some of these metals become available to bind to hemoglobin, and are then in a position to catalyze the oxidation of hemoglobin even after it is chromatographed.

The removal of trace metals by strong chelating agents does not affect the oxygenation or dissociation of hemoglobin. It is therefore a harmless way to drastically stabilize hemoglobin. This method should therefore make it possible to perform very refined experiments on the binding of ligands to hemoglobin.

3. Ethylenediaminetetraacetic acid regulates the oxygenation of hemoglobin. One mole of EDTA is bound to a tetramer of hemoglobin in low salt at concentrations higher than those necessary to maximally inhibit the autoxidation. This binding of EDTA was shown to produce a drastic reduction in the oxygen affinity of hemoglobin.

In the erythrocyte and even in hemolyzed cells the oxygen affinity is much lower than for purified hemoglobin. A low oxygen affinity is physiologically necessary for the effective release of oxygen from hemoglobin. In recent years it has been shown that the oxygen affinity can be regulated by 2,3-diphosphoglyceric acid and to a lesser extent by other organic phosphates which are present in the erythrocyte in high concentrations. The mechanism for lowering the oxygen affinity by these substances is thought to involve the binding of one molecule of these phosphates in the crevice between the β-subunits of hemoglobin. There is a change in the structure of this crevice when hemoglobin is oxygenated and the binding of these molecules to several positive amino groups sticking into this crevice is thought to stabilize the deoxygenated conformation.

A comparison of the structure of EDTA and 2,3-diphosphoglyceric acid indicates a striking similarity in the arrangement of the negative groups. EDTA, therefore, most likely functions as a regulator of the oxygen affinity in the same way as 2,3-diphosphoglyceric acid.

Significance to Bio-medical Research and to the Program of the Institute: The purpose of hemoglobin is to serve as an oxygen carrier to the cells. The study of hemoglobin thus elucidates a vital function of organisms. The role of low molecular weight substances is of particular interest because of the influence which a change in the concentration of some such substance can have on the functioning of hemoglobin. The changes in the properties of hemoglobin which seem to occur in the aging erythrocyte and even in the aging organism may be simply related to a change in the concentration of some such substance in the erythrocyte.

Proposed Course of Project: (1) We plan to continue our study of small molecules which influence the functioning of hemoglobin. The role of divalent metal ions which bind tightly to hemoglobin is of particular interest. (2) With the help of metal complexing agents, we intend to perform very refined

studies on the oxygenation of hemoglobin. (3) It is planned to extend our studies on horse hemoglobin to human hemoglobin. (4) We would like to compare the low molecular weight substances present in erythrocytes of various ages and see how this correlates with the binding properties.

Honors and Awards: None

Publications: None

Serial No. HD-GMA6(1)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mispairing of Nucleotide Bases

Previous Serial Number: None

Principal Investigators: G. Eichhorn (5% time)
J. Pitha (5% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years

Total:	0.35
Professional:	0.10
Others:	0.25

Project Description:

Objectives: (1) To determine the conditions under which mispairing of the complementary bases occurs between polynucleotide strands in order (2) to understand the ways in which misreading of the genetic code can occur. (3) To gain information about the mechanism of interferon induction by polynucleotides.

Methods Employed: It is known that metal ions of the wrong type or in the wrong concentration can bring about the misreading of the genetic code in protein synthesis. Since metal ions have profound effects on the structures of polynucleotides, it is likely that metal ions can induce the formation of hydrogen bonds between bases other than those normally produced in the Watson-Crick structure of DNA. If such mispairing occurs during protein synthesis, it will cause errors in the reading of the genetic code.

Mispairing of nucleotide bases can be determined by studying the effect of various conditions, e.g. the presence of different metal ions, on the binding between a homopolynucleotide and a heteropolynucleotide containing some bases complementary to those on the homopolymer and others that are not. The extent of mispairing can be measured by (a) stoichiometric studies on the combination between the two polynucleotides and (b) kinetic studies to identify non-paired regions by attack of endonuclease specific for single strands.

Major Findings: Preliminary studies on the effect of Mg(II), Mn(II), and Ni(II) on the interaction of poly I with poly UC indicate that the normal stoichiometry observed with Mg(II) is changed in the presence of Mn(II) and Ni(II). Even at low concentrations of polymers (10^{-4}M/P) divalent ions cause some aggregation and special procedures were devised to obtain reliable spectral results. The findings are very preliminary and subject to confirmation.

Significance to Bio-medical Research and the Program of the Institute: The accumulation of errors that would be produced during protein synthesis by mispairing of nucleotides of the kind being studied would lead to deleterious effects in an organism, according to one of the more popular aging theories. Thus the study constitutes a chemical model for a theory of aging. It is also of interest in the study of interferon induction, since the modification of polynucleotide structure by metal ions may lead to a better design of interferon inducers.

Proposed Course of Project: As indicated under Methods.

Honors and Awards: None

Publications: None

Serial No. HD-GMA7
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Biophysics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Influence of Aging and Metal Ions on Collagen Fiber Stability

Previous Serial Number: Same

Principal Investigator: G. L. Becker

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Project Description: This project has been discontinued

Publications: None

Serial No. HD-GMA8
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Biophysics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Elastolytic Enzymes and Elastin in the Aging Rat

Previous Serial Number: Same

Principal Investigator: W. A. Loeven

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.25
Professional:	0.75
Others:	0.50

Project Description:

Objectives: The objective of this project is to determine the temporal changes throughout the life span of rats in the susceptibility of elastic tissues, heart, lung, and aorta to elastolytic enzymes. These findings will supplement previous studies on changes with age in concentrations of pancreatic elastolytic enzymes, serum elastoproteinase inhibitor and elastin concentration in these tissues.

Methods Employed: The susceptibility of unfixed and formalin-fixed cryostat sections (± 10 micron thick) of rat aorta and lung to elastolytic enzymes was studied with histo-enzymatic techniques using resorcin-fuchsin as an elastic tissue stain. The effect of age on mechanical properties of aorta was studied by measuring the time to break of rings of aorta during incubation with elastolytic enzymes (using a constant load of 20 g.).

Major Findings:
Susceptibility of aging lung and aortic tissue of female Wistar rats to elastolytic enzymes.

a. Fixed lung tissue. Although no age changes in susceptibility of parenchymal lung elastic tissue to purified porcine pancreatic elastoproteinase (the proteolytic component) could be observed, the elastic layers around the alveoli and small blood vessels were less susceptible to enzyme treatment in young (1-8 months old) animals than in older mature rats. No age changes in rats between 12 and 24 months of age were found. It was observed that lung elastic tissue from rats suffering of pneumonia was easier

attacked by elastoproteinase than healthy lung tissue.

b. Fixed and unfixed aortic tissue.

1. Large differences were found between the susceptibility to elastoproteinase of fixed and unfixed tissue: (i) no age effect was observed for the susceptibility of interlamellar elastic fibers in fixed tissue to the enzyme; (ii) in unfixed tissue, however, an age effect was present for both the lamellar and interlamellar elastic fibers (tissue from 1-12 months old rats showing less susceptibility than tissue from old rats: no age effect observed in rats 12-36 months old); (iii) under the experimental conditions of the assay, the thick lamellar rings of elastic tissue in fixed aorta was not solubilized.

2. The susceptibility of elastic tissue to the elastomucases (mucolytic elastase components) gradually decreases with age in the fixed tissue of 1-24 months old rats, suggesting that there is a change in structure of aortic elastic fibers in the older rats, not showing when only elastoproteinase is used in the assay.

3. The results of the experiments on the effect of elastolytic enzymes on mechanical properties of fresh rat aorta also indicate a decreasing effect of the enzymes with age. When plotting time to break the aorta ring under a constant load of 20 g against age, positive regression lines were obtained for all the enzymes investigated. However, due to the large standard deviation in each age group studied, the correlation coefficient was only statistically significant when the aortic rings were incubated with elastoproteinase + elastomucase Em-I.

Significance to Bio-medical Research and the Program of the Institute: These findings provide information necessary to develop relationships between biochemical and biophysical properties and morphological aspects of aging elastic tissue. The enzymes of the elastase complex, the chemical structure and mechanical properties of elastic tissue, such as blood vessels and lung, have been partially correlated during the aging process.

Proposed Course of Project: The study of the age-relationship of mechanical properties of rat aorta and of the susceptibility of elastic tissue to elastolytic enzymes will be continued.

Honors and Awards:

Dr. Loeven was invited to present two lectures at the Symposium on Proteolysis and Pulmonary Emphysema, organized by the City of Hope Medical Center and held in Pasadena on January 4-6, 1971: "Elastolytic Activity in Blood" and "Elastolytic Enzymes and Lung Elastic Tissue".

Publications:

Loeven, W. A.: Effect of Hypophysectomy of Female Rats on the Activity of Pancreatic Elastolytic Enzymes and the Elastin Content of Heart, Lung and Aorta, J. Endocrinol. 48, 401407 (1970).

Serial No. HD-GMA9
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Biophysics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Aging Mechanisms in Soft Connective Tissue Studied Through the Use of the Healing Wound

Previous Serial Number: Same

Principal Investigator: Michael D. Sussman

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: With increasing animal age marked changes occur in the mechanical and chemical properties of connective tissue. These changes are assumed to result from an increased intermolecular cross-linking of collagen. This increased stabilization of the collagenous network with age could come about through either the formation of an increased number of enzyme-dependent cross-links or greater stabilization of the bonds already present. Alternatively, the molecules may become more tightly aggregated as a function of the passive establishment of non-covalent intermolecular links whose formation occurs slowly as a function of time. One method of answering this question is to induce the formation of new connective tissue in animals of different age groups, and to see if any differences in physical-chemical properties can be detected. These differences, if present, would indicate the presence of active cellular mechanisms that induce the "aging" of the newly formed connective tissue.

Methods Employed: The model system for study was the healing skin wound. Extensive refinement of existing techniques, and development of new techniques and equipment was necessary in order to detect the sorts of differences which were anticipated. Incisions were made and closed in a standard fashion in the dorsal skin of young (8 month) and old (18 month) male rats. Groups were sacrificed after 2, 5, 8, and 13 weeks of healing and the skin was removed and prepared for testing in a controlled environment of 90% humidity (to prevent evaporational water loss and consequent change in the

physical properties of specimens). Strips of skin of known dimension, with their long axis perpendicular to the wound line, were stamped and physical tests were done with an Instron Tensiometer. This device allows one to apply a constant strain to a specimen and simultaneously record the resultant stress as a continuous record. A specially modified Instron extensometer was directly affixed to the specimens during testing, and gave measurements which closely approximated the extension of the isolated wound tissue. Thickness of the incisions was measured with a Starrett dial indicator. Breaking strength, tensile strength, and extension at break were measured on all specimens. Specimens were tested at strain rates of 2% per minute and 100% per minute.

Major Findings: (1) It has been found that varying the strain rate has a profound effect on the tensile strength of the specimens, the strength at the lower strain rate being significantly less than at the fast rate. Varying the strain rate, however, has no detectable effect on extension achieved at rupture. These results are consistent with results of other investigators and are based on the viscoelastic nature of skin.

(2) Although the tensile strength of wounds of animals in both age groups were similar, the breaking strength of the wounds from younger animals was significantly greater at 8 and 13 weeks. This difference results from a greater mass of scar tissue present in the younger animal, which is also reflected in the increased thickness of the wounds of the younger animals. The extension at break of the younger animals was significantly higher, indicating a more compliant tissue present in the regenerate of the younger animal. This indicates that there are cellular mechanisms in the older animal which induce its regenerated tissue to assume at least one of the characteristics associated with aging — increased stiffness.

Significance to Bio-medical Research and the Program of the Institute: A standard method for the handling and testing of healing wounds has been established which yields reproducible information reflecting basic metabolic and structural properties of these wounds. Certain biomechanical characteristics of healing wounds have been documented. It has been shown that old rats produce less mass of collagenous scar tissue in the healing of a surgical incision, and this tissue in the older animals is somewhat stiffer than that produced by young animals. Whether the quantitative difference is due to fewer cells producing collagen or a decreased amount of collagen produced per cell is not yet known.

Proposed Course of Project: Data will be collected and papers will be prepared for publication.

Honors and Awards: None

Publications: None

Serial No. HD-GMA10
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Biophysics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Enzymes of the Elastase Complex and their Inhibitors in Blood and Connective Tissue.

Previous Serial Number: None

Principal Investigator: W. A. Loeven

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.15
Professional:	0.25
Others:	0.90

Project Description:

Objectives: If the enzymes of the elastase complex and their inhibitors have a role in the aging processes of elastic tissue in heart, lung, and vessel wall, it must be assumed that these enzymes produced in the pancreas are transported to the connective tissues via the circulation, or that there must be other sources of the enzymes in the body. The presence of elastolytic enzymes has been demonstrated in blood (serum, platelets and leukocytes) and in extracts of bovine aorta, but the validity of some of the methods used has been questioned. This project deals with (i) identification, isolation and characterization and (ii) refinement of assays for routinely measuring enzyme and inhibitor activity in blood.

Methods Employed: Several types of elastin preparations are used as substrates for measuring elastolytic activity: unstained elastin, DNS-elastin (elastin covalently bound to dimethyl-amino-naphthalene sulfonic acid) and bromo-acetate-treated elastin (this treatment results in introduction of more carboxyl groups into elastin: higher susceptibility of the substrate to elastolytic enzymes). Synthetic substrates (mostly substituted alanine esters) were also used to measure esterolytic activity of elastoproteinase. Elastoproteinase inhibitor capacity of serum and isolated serum fractions was measured by previously published methods.

Major Findings:

1). Elastoproteinase in serum: the conclusion was drawn that if free elastoproteinase is present in serum, its concentration must be extremely low (less than 10 mμg/ml normal, healthy serum or plasma).

2). Elastoproteinase in platelets: the presence of elastolytic activity as reported by Robert et al (1969) is highly questionable. No enzyme activity could be detected in carefully prepared platelet suspensions using DNS-elastin or acetyl-(l-ala)3-MeOH as substrates; only in less carefully prepared suspensions was low activity measured, probably due to contamination with leukocytes.

3). The recent finding of elastolytic activity in the granules of polymorphonuclear leukocytes by Janoff has been confirmed by us using the substrates mentioned under 2). Enzyme kinetic studies of the leukocyte-enzyme seems to show that its reaction mechanism differs from that of pancreatic elastoproteinase.

4). Elastoproteinase inhibitor in serum: although several authors support the idea that there is only one inhibitor for both trypsin and elastoproteinase in serum, we have recently prepared a fraction (alpha2-globulins) in horse serum that inhibits the proteolytic activity of elastoproteinase and not that of trypsin. Using the same column chromatography technique for human serum was unsuccessful in separating the trypsin and elastoproteinase inhibitors.

Significance to Bio-medical Research and the Program of the Institute: This research provides data on the sources of elastolytic enzymes and inhibitors in the body, their isolation, purification and characterization, their concentration and site of elaboration, and the damage to elastin-containing tissues as a function of age.

Proposed Course of Project: Purification and characterization of the leukocyte-enzyme and the elastoproteinase inhibitor will be continued. Attempts will be made to detect elastolytic activity in connective tissue by means of more sophisticated methods now available.

Honors and Awards: None

Publications: None

Serial No. HD-GMA11

1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Biophysics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Control of Metabolism in the Central Nervous System

Principal Investigators: Bertram Sacktor
Meyer S. Hochman

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.1
Professional:	1.0
Other:	0.0

Project Description:

Objectives: Alterations in the control mechanisms in cerebral metabolism have been initiated in order to understand the deterioration in CNS function with age. Previous studies have shown that hexokinase is a key enzyme controlling the cerebral glycolytic rate. The major portion of the total brain hexokinase, in contrast to that in most other tissues, is localized in mitochondria. Moreover, specific metabolites, at physiological concentrations, can effect the release or rebinding of the enzyme. The physiological significance of this unique localization and the phenomenon of reversible association-disassociation of hexokinase to the mitochondrial membrane are being investigated as a possible critical control of the intermediary metabolism in the central nervous system.

Methods Employed: Current biochemical procedures for brain mitochondrial isolation and measurements of enzyme activities are used, as applicable.

Major Findings: Brain mitochondria exhibit a Crabtree Effect, i.e., the inhibition of respiration by high concentrations of glucose. The addition of glucose leads to accumulation of glucose-6-phosphate. Since the hexose phosphate is a known solubilizer of the mitochondrial hexokinase, the association of hexokinase to the mitochondria during the inhibitory phase of the Crabtree Effect was measured and it was found that the distribution of the enzyme is not altered under these conditions.

Experiments have been carried out to determine whether oxidative phosphorylation will affect the compartmentation of the mitochondrial hexokinase.

ATP is also a known solubilizer of the enzyme, and this could represent a mechanism for regulating brain glycolysis at the first step by the end-product of the metabolic sequence. Preliminary findings suggest an increased release of hexokinase after State 3 respiration.

Significance to Bio-medical Research and the Program of the Institute: The mechanisms of the biochemical control processes in the central nervous system are being established. This information is fundamental to the understanding of the deterioration of CNS function during the aging process.

Proposed Course: Studies are planned to define further the role of hexokinase in the regulation of cerebral metabolism. Possible age-related changes in the control mechanisms will be explored.

onors and Awards: None

'ublications: None

Serial No. HD-AG23(8)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Intermediary Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigations on Inter- and Intracellular Transport Mechanisms

Previous Serial Number: Same

Principal Investigators: Bertram Sacktor
Sosamma Berger
Russell Chesney

Other Investigators: None

Cooperating Units: None

Man Years

Total:	3.8
Professional:	2.3
Others:	1.5

Project Description:

Objectives: Physiological systems, such as renal function, which are intimately dependent on transport of ions and metabolites across cellular membranes, show marked functional changes with age. The paucity of information on the enzymatic mechanisms for the transport of these vital cell constituents frequently precludes a full explanation of observed alterations. Investigations on the enzymatic basis for the active transport of carbohydrates and ions has been initiated to provide this needed information.

Methods Employed: Modern biochemical procedures including enzyme kinetics and purification, column and paper chromatography, radioisotopes, subcellular fractionation, histochemistry and electron microscopy are used.

Major Findings: The renal brush border, isolated for the first time by a novel procedure, has been characterized further. The major species of neutral lipids, phospholipids, and glycolipids of the membrane have been identified, after separation by column and thin layer chromatography, and quantitated. Noted are the high concentrations of sphingomyelin and phosphatidyl ethanolamine.

The proteins comprising the brush border have been separated by polyacrylamide gel electrophoresis after establishing a procedure for solubilizing the membrane with retention of enzymatic activities. Bands, representing

trehalase, maltase, leucine aminopeptidase, alkaline phosphatase, trehalose-6-phosphate phosphatase, and Mg^{++} and $Na^{+} + K^{+}$ ATPases, have been identified. Ouabain sensitivity of the $Na^{+} + K^{+}$ ATPase is lost with solubilization of the enzyme. Sensitivity is not restored by the addition of phosphatidyl serine, as has been reported for the brain microsomal enzyme.

The kinetics of the binding of D-glucose to the isolated brush border is being studied since this represents the first step in the transtubular transport of the sugar. Binding has been characterized with respect to time, and concentrations of brush border and glucose. Two kinetically different binding mechanisms have been identified: one, a high affinity site that has a Km of 0.6 μM and is saturated at about 2 μM glucose; the other, a low affinity site that has a Km of 20 mM and is saturated at approximately 40 mM. Binding is inhibited by phlorizin. Inhibition is competetive at the high affinity site but non-competetive at the low affinity site. The binding is reversible. Relative to D-glucose, L-glucose is poorly bound, demonstrating the specificity of the binding mechanism.

Significance to Bio-medical Research and the Program of the Institute: This fundamental information is required to define the biochemical mechanisms of transport, which will then be used in studies of the age-dependent changes in transport systems.

Proposed Course: Studies will continue to elaborate further the mechanism of the active transport of sugar in kidney and intestine.

onors and Awards:

Dr. Sacktor was invited to present the principal lecture on "Mammalian Trehalase" to the V International Symposium on Carbohydrate Chemistry, Paris.

Dr. Sacktor was invited to present a symposium paper on "The Role of Trehalase in Sugar Transport" to a Symposium on "The Biochemistry of the Glycosidic Linkage" in honor of Dr. Luis Leloir, San Carlos de Bariloche.

ublications:

Berger, Sosamma J., and Sacktor, Bertram: Isolation and characterization of brush borders from rabbit kidney. J. Cell Biol. 47: 637-645, 1970.

Benson, Robert L., Sacktor, Bertram, and Greenawalt, J. W.: Studies on the ultrastructural localization of intestinal disaccharidases. J. Cell Biol. 48: 711-716, 1971.

Sacktor, Bertram, and Wu, Niou-Ching: Studies on the spatial relationship between intestinal disaccharidases and the phlorizin-sensitive transport of glucose. Arch. Biochem. Biophys., in press.

Serial No. HD-AG20(8)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Intermediary Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Mechanism of the Decrease in Maximal Work Performance During Senescence

Previous Serial Number: Same

Principal Investigators: Bertram Sacktor
Douglas Reed
Niou-Ching Wu
Yoshio Shimada (EOD 9/70)
Shri Shukla

Other Investigators: Albert Lehninger
Ernesto Carafoli

Cooperating Units: Department of Physiological Chemistry, Johns Hopkins University, School of Medicine, Baltimore, Maryland

Man Years

Total:	5.7
Professional:	4.2
Others:	1.5

Project Description:

Objectives: Muscular work performance decreases markedly during aging. In a model system, the flight of insects, the duration of flight, and wingbeat number and frequency decline with age. Previous studies have shown that muscular activity is dependent on the control of carbohydrate metabolism. The mechanisms of regulation at different critical control loci are examined in order to determine the basis for the decrement in excitation-contraction-bioenergetic coupling that is found during the aging process.

Methods Employed: Procedures and techniques of modern biochemistry are modified or developed, as applicable.

Major Findings: Flight muscle mitochondria from senescent blowflies, Phormia regina, (25-35 days old) have a significantly lower State 3 rate of respiration (the maximal rate of O_2 uptake obtainable in the presence of excess substrate, ADP, Pi, and oxygen) than those from mature (7-10 days old) flies. Neither the "resting" State 4 rate nor the ADP/O ratio is

perturbed by age. The decreased State 3 rate is obtained with the two physiological substrates of these mitochondria, α-glycerophosphate and pyruvate + proline, indicating that the decrement in oxidative capacity is localized in the respiratory chain. The importance of the additions of metal chelating agents and serum albumin to preparative and reaction media was characterized in greater detail and the findings suggest the action of endogenous phospholipase in altering mitochondrial stability.

Correlated ultrastructural studies on the flight muscle reveal an unique degradation of the mitochondria of aging blowflies. The inner membrane of the mitochondria undergoes a transformation and appears as myelin-like whirls. At the locus of deformation, the mitochondrion becomes less turgid and the cytosol, filled with glycogen, intrude. The whirls increase in size and number and eventually replace the normal cristal configuration. Histochemical studies at the electron microscope level show that the respiratory chain, measured by cytochrome oxidase, is not functional in that portion of the mitochondrion showing structural damage. Lysosomal activity is not associated with this morphological alteration; acid phosphatase activity, measured histochemically at the resolution of the electron microscope, is absent. These structural changes in mitochondria from aged flies are seen in the muscle in situ as well as in the isolated mitochondria that were used in the biochemical studies noted above.

A study of the ultrastructural localization of the enzyme trehalase in flight muscle has been completed. Approximately 65% of the total activity sediments with a low speed pellet; 25% of the activity in the homogenate is soluble. The activity in the particulate fraction resides in the mitochondria. Isolated mitochondria have been fractionated by different procedures and the distribution of trehalase has been compared to the distributions of α-glycerophosphate dehydrogenase, hexokinase binding, malic dehydrogenase, and adenylate kinase, representing the inner and outer membranes, the matrix, and the space between the outer and inner membranes of the mitochondria, respectively. Trehalase is clearly separated from the enzyme markers of the matrix and the space between the two membranes. Its distribution closely resembles that of α-glycerophosphate dehydrogenase rather than that of hexokinase binding. Thus, these findings support the tentative conclusion that in blowfly flight muscle the mitochondrial trehalase is localized on the inner membrane. If the permeability of trehalose in mitochondria is the same as that of sucrose, then it is suggested that trehalase is situated on the outside of the mitochondrial inner membrane. The evidence to-date favors the view that the soluble trehalase activity in muscle homogenates represents a muscle enzyme distinct from the mitochondrial trehalase.

A study of the mechanism of the control of glycogenolysis has continued. We previously suggested that in flight muscle initiation of contraction is coupled to glycogenolysis by a conversion of phosphorylase b to a, triggered by the activation of phosphorylase b kinase by calcium ions and inorganic phosphate (Pi). It is now found that the kinase is activated by preincubation with Pi, in the absence of phosphorylase b and ATP, indicating a novel mechanism. A plot of activation as a function of Pi concentration shows allosteric type kinetics, with saturation at 50 mM. Mg^{++} (5 mM) is

required for the maximal effect of Pi; activation by a given concentration of Pi depends on the concentration of Mg^{++}. Activation of the kinase by Ca^{++} is in its physiological range, 10^{-7} M. Ca^{++} increases the Vmax and affinity for phosphorylase b. Pi and Ca^{++} act cooperatively, the combination yields rates far greater than the sum of the two activators, separately. pH optima for the actions of Pi and Ca^{++}, separately and together have been determined. The kinase is activated many-fold without any change in the ratio of activity at pH 6.8 and 8.2, a value commonly used in studies in mammalian tissues of the effects of cyclic AMP and epinephrine on the kinase and, in turn, on glycogenolysis. The mechanism of kinase activation discovered in flight muscle is being examined in rabbit skeletal muscle.

A study of the interaction of Ca^{++} with flight muscle mitochondria has been completed. Blowfly mitochondria differ markedly from mammalian mitochondria in their interaction with Ca^{++}. State 4 respiration of blowfly mitochondria is not stimulated by Ca^{++}, nor does Ca^{++} induce a reversible shift in the oxidation-reduction state of cytochrome b. Nevertheless, Ca^{++} is accumulated by respiring blowfly mitochondria, but with a low efficiency; the Ca^{++}: $\sim$ accumulation ratio is about 0.6 with pyruvate plus proline, and about 0.2 with glycerol 1-phosphace, compared with values of about 2.0 observed in mammalian mitochondria. Ca^{++} uptake is blocked by respiratory chain inhibitors and by uncouplers, but not by oligomycin. The presence of permeant anions in the medium is essential for Ca^{++} uptake by blowfly mitochondria. At high levels of Ca^{++}, phosphate supports Ca^{++} uptake better than acetate, but at low Ca^{++} levels, phosphate and acetate are equally effective. Since acetate penetrates blowfly mitochondria passively it is probable that respiration-coupled uptake of Ca^{++} in blowfly mitochondria must be accompanied by passive entry of matching anions. The accumulation of Ca^{++} by blowfly mitochondria is very slow and does not exhibit saturation kinetics; moreover, no high affinity Ca^{++} sites characteristic of a specific Ca^{++} carrier could be detected. For these reasons it is concluded that the respiration-dependent accumulation of Ca^{++} in blowfly mitochondria is not mediated by a specific carrier, but occurs through slow, concentration-dependent, physical diffusion through the membrane, in response to an electro-chemical gradient generated by electron transport. The number of low affinity Ca^{++}-binding sites is surprisingly low, about one-tenth of that found in mammalian mitochondria, and has been related to the peculiar phospholipid composition of these mitochondria. The findings described strongly suggest that the mitochondria of blowfly flight muscle do not actively segregate Ca^{++} at the low concentrations at which it is presumed to occur in the sarcoplasm *in vivo*.

Significance to Bio-medical Research and the Program of the Institute: The biochemical mechanisms for controlling the metabolism of muscle are being established to provide the fundamental information needed to understand the decrements in the excitation-contraction-bioenergetic processes with age.

Proposed Course: Studies are planned to define further the mechanisms of regulation and the changes in structure and function of mitochondria with age.

Honors and Awards:

Dr. Sacktor was invited to present seminars at The Pennsylvania State University Medical School, Hershey, Pennsylvania, and The Institute for Cancer Research, Philadelphia, Pennsylvania.

Dr. Sacktor was invited to present Symposium lectures at the Annual Meeting of the Entomological Society of America and the Middle Atlantic Regional Meeting of the American Chemical Society.

Publications:

Childress, Charles C., and Sacktor, Bertram: Regulation of glycogen metabolism in insect flight muscle. Purification and properties of phosphorylases in vitro and in vivo. J. Biol. Chem. 245(11): 2927-2936, 1970.

Smith, David S., and Sacktor, Bertram: Disposition of membranes and the entry of haemolymph-borne ferritin in flight muscle fibers of the fly Phormia regina. Tissue and Cell 2(2): 355-374, 1970.

Hansford, Richard, and Sacktor, Bertram: Oxidative metabolism of insects. In Florkin, M., and Scheer, B. (Eds.): Chemical Zoology V. London and New York, Academic Press, 1971, pp. 213-247.

Carafoli, Ernesto, Hansford, Richard G., Sacktor, Bertram, and Lehninger, A. L.: Interaction of Ca^{++} with blowfly flight muscle mitochondria. J. Biol. Chem. 246(4): 964-972, 1971.

Reed, W. Douglas, and Sacktor, Bertram: Localization of trehalase in flight muscle of the blowfly Phormia regina. Arch. Biochem. Biophys., in press.

Serial No. HD-AG19(8)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Intermediary Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Mechanism of Action of Endocrines in Regulating Intermediary Metabolism and Homeostasis

Previous Serial Number: Same

Principal Investigators: Bertram Sacktor
Bernard Bulos

Other Investigators: None

Cooperating Units: None

Man Years

Total:	2.5
Professional:	1.1
Others:	1.4

Project Description:

Objectives: Effective understanding of age-related alterations in the function of endocrines in regulating and controlling intermediary metabolism and homeostasis is largely dependent on knowledge of the mode of action of the hormone at the cellular and enzymatic levels. This investigation aims to provide information on the mechanism of the thyroid-induced specific synthesis of the mitochondrial α-glycerophosphate dehydrogenase and on the underlying basis for differences in response of peripheral tissues to the hormone. The specific induction of the enzyme by thyroid represents an excellent model system to test the hypothesis that various components of the cellular machinery for protein synthesis are altered during aging. A cognate objective is to answer the fundamental questions as to the site of synthesis of a mitochondrial enzyme, how this protein becomes incorporated into the mitochondrial cristae *in vivo* and the identity of the constitutive and induced enzyme.

Methods Employed: Up-to-date biochemical techniques, developed in these laboratories or in the current literature, are used.

Major Findings: Previous work has shown that the specific activity of the mitochondrial α-glycerophosphate dehydrogenase of rat liver is dependent on the thyroid state of the animal and that the specific activity of the enzyme in the hypo-, eu- or hyperthyroid rat is not altered as a function

of age. Thus, the hyperthyroid animal is fully able to synthesize the dehydrogenase in response to thyroxine (T_4) regardless of the age of the animal. This study, however, was based on the maximal obtainable induction of the enzyme since the animals were maintained on a diet containing T_4 for 14 days. It was considered possible, however, that the rate of induction of the enzyme, but not its maximal extent of induction, might be altered in senescent rats as compared to adults. For this reason, adult (12 month old) euthyroid rats were injected with a single dose of triiodothyronine (T_3) and the specific activity was determined 6, 12, 18, 24, 36, 48, and 72 hours after injection. Maximum activity was observed at 36 hours after injection and represented a 2-fold stimulation in specific activity. Increasing the dose of T_3 did not increase the maximal attainable specific activity. This limited stimulation in these adult rats would make comparison of the rate of enzyme synthesis with senescent rats very difficult. Surgical removal of the thyroid to increase the extent of the response to T_3 had to be abondoned because of the above average mortality rate in 24-27 month old animals after thyroidectomy. The present approach is to reduce the level of α-glycerophosphate dehydrogenase by maintaining rats on a diet of 0.3% thiouracil. After a 4-week period, the specific activity of the enzyme decreased from 12-14 to 4-5 units/g protein in mitochondria of both adult and senescent rats. Preliminary experiments indicate that TU-fed rats respond to T_3; a single injection yielding mitochondria with about 35 units, an 8-fold increase over control animals.

In order to compare the hormonally induced enzyme with the constitutive form of the enzyme, the solubilization and purification of the mitochondrial inner membrane bound enzyme is required. A technique using snake venom phospholipase A showed promise and a reliable and rapid assay for the phospholipase in Naja naja venom was developed. Asolectin, a mixture of soybean phospholipids, is used as the substrate instead of the more expensive, natural substrate L-α-lecithin. Phospholipase A activity of snake venom, monitored by hydroxamate formation, obeys Michaelis-Menten kinetics and is proportional to time and venom concentration. The pH profile of activity shows a broad plateau from pH 7.0 to 9.5 and a shoulder between pH 5 and 6.

Solubilization of the dehydrogenase with detergents under very carefully defined conditions, followed by polyacrylamide gel electrophoresis, resulted in separation of protein bands with retention of some enzyme activity. Three bands with α-glycerophosphate activity were observed in mitochondria from T_3 injected animals. Whether these bands represent differences between the induced and native enzymes or represent isozymes of both is currently under investigation.

Significance to Biomedical Research and the Program of the Institute: This study is testing the hypothesis that the rate of synthesis of a specific enzyme in response to an inducer is altered in aging.

Proposed Course: The rate at which thyroid hormone induces the specific synthesis of an enzyme will be examined in animals of different ages. The constitutive and induced forms of the enzymes will be compared further.

Honors and Awards: None

Publications:

Bulos, B., Sacktor, B., Grossman, I. W., and Altman, N.: Thyroid control of mitochondrial α-glycerolphosphate dehydrogenase in rat liver as a function of age. J. Gerontol. 26(1): 13-19, 1971.

Bulos, B., and Sacktor, B.: An assay for phospholipase A activity of snake venom with the use of asolectin as substrate. Anal. Biochem., in press.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971
Gerontology Research Center
Laboratory of Cellular and Comparative Physiology

During the past year, the Laboratory of Cellular and Comparative Physiology investigated lysosomal activity during growth and aging, and the effect of environment on the rate of aging. Histochemical studies were initiated in the rotifer in an attempt to understand the nature of Inclusion Bodies, cellular organelles which were found to increase greatly in concentration in several organs of old rotifers. Earlier histochemical studies have shown that the age-dependent distribution of two lysosomal enzymes, acid phosphatase and β-glucuronidase, was consistent with the age-dependent alterations in Inclusion Body concentration. A histochemical study of a third lysosomal enzyme, E600 resistant esterase, has been carried out. The activity of this enzyme was found to increase in the stomach, ovaries and dorsal gastric gland of old rotifers, a pattern which is also consistent with the age-dependent changes in Inclusion Body concentration. Thus the histochemical studies strongly suggest that Inclusion Bodies are some form of lysosomes. Since the studies described above have implicated lysosomes in the aging process in rotifers, an attempt was made to alter life span by growing the animals in the presence of substances known to alter the stability of lysosomal membranes. To date, the effects of hydrocortisone and chloroquine, both stabilizers of lysosomal membranes, have been tested. Chloroquine was found to increase longevity, primarily in very old rotifers. This substance doubled the further life expectancy of rotifers at the age of 50% survivorship. Old, chloroquine treated animals were much more active than their untreated controls. Hydrocortisone had no effect on longevity.

Little is known regarding the fundamental structural and functional principles underlying the activities of highly differentiated, non-renewable cells as they may be correlated with changes in tissue and organ function with age. A recently completed ultrastructural study of myocardial cells of the left ventricle of rats aged 6, 8, 10, 12, 18, 24, 27, and 33 months has clearly shown that marked changes begin to occur at about 18 months and continue through 33 months. The ultrastructural changes within the myocardial cells included: (1) increases in the number of residual bodies, which corresponded to lipofuscin or age pigment viewed with the light microscope; (2) increases in primary lysosomes in the mitochondrial cones at the nuclear poles and in mitochondrial rows between the myofilaments; (3) mitochondrial degradation by lysosomal activity; (4) increases in the number and changes in the nature of the lipid droplets and (5) partial and complete degradation of lipid droplets by lysosomal activity and autoxidation. These changes within the myocardial cells precede any reported measurable physiological or biochemical decrements in cardiac capacity with age by at least 6 months. It is clear from these studies that degradation of mitochondria to residual bodies by lysosomal activity occurs. This takes place in cells from old (18-33 months) animals at a rate which exceeds their capacity to extrude the residue. However, certain properties of old cells must account for increased lysosomal activity which leads to the accumulation of these residual bodies.

During molting, the crayfish does not feed and the organic and mineral salts from the old skeleton are broken down, resorbed and deposited in the soft tissues. Lysosomes appear to be involved in digestion of cell components such as phospholipids and sterols which are reutilized in the synthesis of the newly mineralizing matrix.

Previous ultrastructural studies on aging Campanularia flexuosa suggested increased hydrolytic enzymatic activity on the basis of lysosomal degradation of certain cellular organelles. Biochemical studies to quantify these changes have shown the following: total acid phosphatase and free activity (secondary lysosomal activity) doubled with age in hydranths cultured at 10°C. When these activities were expressed per unit protein or DNA, such increments were not demonstrable due to increases in protein and DNA concentrations with age, which resulted from continued cell division and replacement of other cell types not showing lysosomal activity. It was shown that different isoenzymes of acid phosphatase, reactive at different optimal hydrogen ion concentrations, depend upon the environmental temperature as well as the season of the year. For example, changes in the number and activity of isoenzymes of acid phosphatase occurred throughout development, adult life, and regression of hydranths at 10°C during mid-winter.

On the basis that limitations in the use of genomes retard aging and increase life span in animals subjected to protein deprivation, C_{57}BL/6J mice were offered diets which contained either 4% or 26% casein ad libitum and several enzymatic activities of heart, liver and kidney were measured following 2 and 6 months of feeding. The activities of the majority of enzymes studied were lower in the animals fed the 4% as compared to the 26% casein diet. The exceptions were high levels of the activities of cathepsin and alkaline phosphatase of tissues of animals fed the low protein diet. It should be pointed out that these latter enzymes have a pattern of change with age opposite that of the majority--i.e., the activities decrease rather than increase during early life. After 15 months of feeding it was observed that most of the enzymatic activities in controls had reached maximal values and had started to decline whereas those in the experimental group continued to increase toward these maximum levels. In addition, the changes in extractibility of collagen from skin also indicated that the mice fed the low protein diet were like chronologically younger animals. This conclusion is also supported by the higher tensile strength of tail tendons in the dietarily restricted than control group. Other data which support the hypothesis that dietary restriction retards aging by limiting the use of the genome are the lower rates of protein turnover in heart and skeletal muscle of the animals fed the 4 than those fed 26% casein diet.

It is well established that poikilothermic animals exposed to high temperatures have a shortened life span. Recent studies on the hydranth, Campanularia, have indicated another dimension in the control of longevity of such organisms, i.e., in spite of constant environmental conditions in the laboratory, there are seasonal fluctuations in life span which seem to be related to alterations in the environmental temperature of their natural habitat. For example, the shortest life span in the laboratory occurred when the water temperature at Woods Hole (the natural habitat) was highest

and, conversely, the longest life span occurred when the water temperature was lowest. At present, no explanation can be offered to account for this biologic phenomenon.

An unknown nucleotide has been isolated from erythrocytes of cold-exposed and hibernating hamsters. The concentration of this nucleotide, based on measurement of total phosphate, increased during hibernation. Although its spectral characteristics are similar to inosine phosphate, several experiments including thin layer chromatography, indicated that it was not identical to IMP, IDP or ITP. Since inosine and adenosine, when used as supplements to blood preservatives, increase the viability of erythrocytes in vitro, it is important to determine whether this inosine-like compound is responsible for the longer life span of erythrocytes in hibernating hamsters than in controls.

Serial No.: HD-AG35

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The possible involvement of a translational mechanism for gene repression in differentiation and aging.

Previous Serial Number: Same

Principal Investigator: D. D. Hendley

Other Investigators: None

Cooperating Units: None

Project Description: This project has been discontinued.

Publications: None

Serial No.: HD-CCP4(c)
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The composition and function of lipofuscin particles

Previous Serial No.: Same

Principal Investigators: D. D. Hendley

Other Investigators: None

Cooperating Units: None

Project Description: This project has been discontinued.

Publications: None

Serial No.: HD-AG33 (8)
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Changes in the dynamics of degradation and renewal of cellular components during development and aging

Previous Serial Number: Same

Principal Investigator: P. H. Gold

Other Investigators: R. A. Menzies

Cooperating Units: Department of Biochemistry
Louisiana State University
New Orleans, Louisiana

Project Description: This project has been discontinued.

Publications: Gold, P. H., and Menzies, R. A.: The turnover of mitochondria in a variety of tissues of young adult and aged rats. J. Biol. Chem. In press.

Serial No.: HD-AG12

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effect of age on metabolism of 2-^{14}C acetate in the rat

Previous Serial Number: Same

Principal Investigators: D. C. Moses
M. J. Yiengst
C. H. Barrows

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Project Description: This project has been discontinued.

Publications: None

Serial No.: HD-CCP7(c)
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The effect of age on human leucocytic cells *in vitro* and *in vivo*

Previous Serial Number: Same

Principal Investigator: J. A. Silbert (100%)

Other Investigators: None

Cooperating Units: Melloy Laboratories
National Cancer Institute
Springfield, Virginia

National Cancer Institute
Viral Leukemia and Lymphoma Branch
Bethesda, Maryland

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: After careful evaluation of the possible experimental designs for evaluating DNA repair mechanisms, it became evident that none were sensitive enough to detect differences in DNA repair less than 15-20% of the control value. Therefore, it was felt that a negative result would be inconclusive and the experiment was not worth the effort. This was born out by the experiments of Dr. Samuel Goldstein (personal communication) who studied DNA repair in senescent cultures of fibroblasts by autoradiography and found no differences from younger control cultures. Emphasis was then shifted to the study of the karyotypic and growth characteristics of continuously propagated human hematopoetic cells in order to determine the differences and similarities of these cells to the human fibroblast in culture.

Methods Employed: Cultures were grown in RPMI 1640 media supplemented with 15% fetal calf serum. The cells were kept in a state of continuous

growth. Careful records were kept of the cell population doublings using a Coulter Particle Counter. Approximately every 10 population doublings, a chromosome preparation was made using a modification of Paul Moorhead's technique. Growth curves for each of the three strains studied were made for four different serum concentrations at the beginning and end of the experiment. Seventy-seven to 111 population doublings accrued during the experiment depending on the culture studied. Fluorescent antibody and electron microscopic studies were performed in order to detect viral infection. Mycoplasma was assayed during the experiment by H.R.C. Inc., Baltimore, Maryland.

Major Findings: (1) Hematopoetic cells do not exhibit any signs of senescence as do fibroblasts over a comparable number of cell population doublings. (2) The karyotype is quite different from that of non-senescent lines of human fibroblasts in that the modal number of chromosomes is always around 46 instead of the triploid number 69 as in fibroblasts. (3) The serum requirements of the hematopoetic cells are almost identical to that of normal diploid human fibroblasts and did not change over the course of the experiment. (4) Two of the three lines tested were positive for a Herpes-like virus. No correlation could be found between the karyotype or growth at different serum concentrations to virus infection.

All hematopoetic cell lines tested by Dr. George Todaro, et al. at Melloy Laboratories contained RNA primed DNA polymerase (reverse transcriptase).

Significance to Biomedical Research and the Program of the Institute: The answer to one question posed in the previous report seems now to be settled. Namely, the phenomenon of senescence in vitro is not a general one for all tissues as the hematopoetic cells are non-senescent and clearly show a number of characteristics at variance with both diploid and aneuploid human fibroblasts. The relation of karyotype to senescence in vitro is a complex issue and a review discussing this topic is in preparation for a chapter "Cellular Senescence and Death" in the book, Pharmacology of the Cell. A manuscript detailing the experiment outlined above is also in preparation. The issue of cellular senescence in vitro is an important one to gerontologists as the possibility of an inherent biological limitation of division potential of dividing cells contributing to the deterioration with age of the organism has been raised. This subject will also be reviewed in "Cellular Senescence and Death."

Proposed Course of Project: This project will be concluded in April 1971. In the interim period further studies of the growth and phenotypic characteristics of the hematopoetic cells will be attempted if time permits.

In addition to the studies of the hematopoetic cells I have obtained, through the courtesy of Dr. William Reichel and Dr. Robert Hay, three strains of fibroblasts from individuals with progeria. These cells will be grown and stocked for the future use of other investigators at the Gerontology Research Center and NIH. In collaboration with Dr. Stuart Aaronson in the National Cancer Institute, these cells are being transformed by SV40 virus in order to establish a permanent line of cells. Preliminary studies indicate that progeria cells are abnormally susceptible to SV40 viral transformation.

Honors and Awards:

Guest Seminars--The Aging of Cell Cultures and its Relationship to Aging in the Intact Organism given in the:

Department of Botany
University of Leeds
Leeds, England
November 21, 1970
Sponsor: H. W. Woolhouse, Chairman

Department of Development Biology
Harvard Medical School
Cambridge, Massachusetts
January 15, 1971
Sponsor: Jerome Gross, Chairman

Scholarship for a course in cell culture for the study of aging by Alton Jones Cell Science Center, Lake Placid, New York--June 21-July 9, 1971

Publications: None

Serial No.: HD-CCP6

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Age changes in organ biochemistry associated with dietary alterations

Previous Serial Number: Same

Principal Investigator: Salvatore Leto (100%)

Other Investigator: Charles H. Barrows, Jr. (50%)

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	2.5
Professional:	1.5
Other:	1.0

Project Description:

Objectives: Nutrition has a well established role in the aging process. This investigation is part of a program which has addressed itself to the question of the contributing role played by nutrition in this biological phenomenon. A clarification of the role of nutrition in senescence is sought by the application of dietary alterations on experimental animals and subsequent examination of selected organs and tissues by biochemical means. Possible related physiological variables are also being investigated in these animals as a function of chronological age. Specifically, this investigation during the fiscal year has included: (1) The continuation of a study of the effect of dietary protein and age on the enzymatic activity of C_{57}BL/6J female mice. (2) The measurement of the following related variables in this group of mice under the above mentioned altered dietary regimen: (a) The effect of age and diet on skin collagen extractability; (b) A collaborative study to determine the effect of diet and age on tail tendon tensile strength; (c) The effect of diet on protein turnover in several organs of these experimental animals; (d) The effect of age and diet on body temperature; and (e) The effect of age and diet on basal metabolism in the intact animal.

Methods Employed: (1) Assay methods have been previously described. (2) The diets used are synthetic diets supplied by General Biochemicals,

Chagrin Falls, Ohio modified to our specifications. (3) Rectal temperature measurements were obtained with a portable Yellow Springs Telethermometer model 44TA using a thermistor probe. (4) Turnover measurements were made using standard tracer techniques. (5) Basal metabolism was determined by spirometry measurements.

Major findings: (1) Effect of age and diet on enzymatic activity. Feeding 4% casein (experimental) vs 26% casein (control) has had little or no effect thus far (60 weeks) on enzymatic activity (heart LDH, MDH, alkaline phosphatase; kidney LDH, MDH); caused a decrease (liver LDH, MDH, cholinesterase succinoxidase; kidney succinoxidase); or an increase (liver alkaline phosphatase, cathepsin; with a rising trend toward an increase in kidney cathepsin which is not significant up to the 60th week). The activity of LDH, MDH and succinoxidase measured in the various tissues has reached a peak and shows a declining trend which appears to be more rapid for the control animals. It would appear that the effects of the imposed restriction are beginning to become more evident as the animals increase in chronological age.

(2) Variables related to protein restriction and age. (a) The effect of age and diet on skin collagen extractability. As reported by others for rats, mouse skin collagen extractability also shows an age-associated decline when fed a normal diet. Dietary restriction appeared to cause a decrease in extractability of skin collagen; however, by the 60th week of restriction the restricted animals have a higher extractability than controls. This is in agreement with some of the enzymatic activity patterns and indicative of a "younger" animal. (b) The effect of diet and age on mouse tail tensile strength. The data parallels that seen with skin extractability; i.e., lower extractability is directly related to higher tensile strength. Therefore the sixty week old animal tail tensile strength for the restricted group is lower, indicative of a "younger" animal. (c) The effect of diet on protein turnover. Liver, kidney, heart and muscle protein turnover were measured by radioisotope techniques. No differences were found in liver and kidney turnover, while heart and skeletal muscle turnover was found to be lower in the experimental animals. This is in agreement with the findings in rats placed on protein restricted diets. (d) The effect of age and diet on rectal body temperature. Protein restriction was seen to be related to a small but significantly lower rectal body temperature. The greatest difference over 24 hour cycles was seen to occur while the animals were resting. During overnight activity periods little or no difference was seen in both groups. This suggests that the restricted animals have the metabolic capacity but at comfortable ambient conditions allow their body temperature to drift lower than controls. Perhaps an energy conserving mechanism is involved. Lowering ambient temperature causes a greater response by the restricted animals and at temperatures below 18°C little difference is seen between control and restricted groups during inactivity periods indicating the restricted animals are capable of the metabolic response and indeed do respond to the temperature stress. (e) The effect of age and diet on basal metabolism. Oxygen consumption measurements in control and experimental animals as well as measurements in animals fed commercial natural diets, show a decrease in oxygen uptake as a function of age. Restricted animals, however, seem to have a higher oxygen uptake per gram of mass than controls.

Measurements are currently under way to determine whether this difference is due to differences in "metabolic size" of the animals rather than total body mass.

Significance to Biomedical Research and the Program of the Institute: Despite marked differences in life spans of many organisms, evidence continues to accumulate indicating that the pattern of senescence may be similar in all organisms. By selecting and studying biochemical systems common to different species or strains within a species, we hope to integrate available data into a unified explanation of senescence. Since nutrition has been shown to affect the longevity of all organisms thus far tested, it is felt that this approach may be fruitful in our search toward elucidating biological aging mechanisms.

Proposed Course of Project: The findings thus far in the enzyme studies do agree with similar studies in other species; however, final conclusions must be reserved until all the data are in. The other variables that have been measured and the findings listed above indicate that indeed nutrition affects many biochemical and physiological systems in an organism. Therefore, it is proposed that this project be continued in order to accumulate data in the above listed variables for the entire life span of the experimental animals.

Honors and Awards: None

Publications: Leto, S., Kokkonen, G. C. and C. H. Barrows: Age changes in various biological measurements of C_{57}BL/6J female mice. J. Geront. 26: 24-27, 1971.

Serial No.: HD-CCP8

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Morphology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ultrastructural changes in the rat myocardial cells during developmental sequences

Previous Serial Number: Same

Principal Investigator: Dorothy F. Travis (40%)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	0.9
Professional:	0.4
Other:	0.5

Project Description:

Objectives: The broad objectives of this program have been directed at understanding basic structural and functional principles underlying the activities of highly differentiated, non-renewable cells as they may be correlated with changes in function at the tissue and organ level during developmental sequences. It has been the specific objective of the present study to investigate the ultrastructural changes in the rat left ventricular myocardial cells throughout an age span of 6-33 months in an attempt to answer the questions relating to the function of lysosomes, the ontogeny of residual bodies, and their possible effects on myocardial function during the ageing process.

Methods Employed: Methods were the same as described in the previous annual report.

Major Findings: The last reported major findings related to a pilot study completed on two age groups of rats: (1) the mature, 8 month old rats and (2) 33 month old rats. The present completed ultrastructural study relates to rats of 6, 8, 10, 12, 18, 24, 27, and 33 months of age.

(A) General structural organization of the rat myocardial cell. For information, see the last annual report.

(B) Ultrastructural changes in the myocardial cell with age. Although the basic findings from the previous pilot study remain unchanged, considerably more information has been added with the study of other age groups.

The present results indicate that marked ultrastructural changes occur in the left ventricular myocardial cells of the rat with age, and that the transitional stage of development at which these changes begin to occur is at about 18 months and continue through 33 months. These changes are most conspicuously reflected in increases in the number of residual bodies, corresponding to the lipofuscin pigment viewed in light micrographs; increases in the number of primary lysosomes in the mitochondrial cones at the nuclear poles and in the mitochondrial rows separating the myofilament masses; mitochondrial degradation by lysosomal activity; apparent increases in the number of lipid droplets, changes in the nature of these, and their partial or complete segregation in autophagic vacuoles for their presumed degradation.

Residual bodies. Our own estimates indicate that there are double the number of residual bodies in the left ventricular myocardial cells of 18-33 month old rats as compared to 6-12 month old animals. These residual bodies were confined exclusively to (1) the mitochondrial cones at the nuclear poles and (2) the mitochondrial rows in the sarcoplasmic clefts separating the myofilament masses. Like electron dense residual bodies observed in other cell types, they are membrane-bounded of variable size, shape and fine structure. Many were surrounded by double unit membranes which in itself was interesting, because the membranes are the same thickness as are mitochondrial membranes and because the size and shape of the residual bodies resemble mitochondria as they are observed in different profiles. Since DeDuve (1963), DeDuve and Wattiaux (1966) have proposed that residual bodies represent undigested residues that result from a dynamic and progressive autophagic process, proceeding from the segregation of cellular components through their lysis to residual material by lysosomal activity, questions relating to the function of lysosomes, the ontogeny of residual bodies, and their possible effects on myocardial function with age are not fully known and are of importance.

Lysosomes. In older animals of 18 through 33 months, increases in primary lysosomes were observed in the nuclear pole zones in the vicinity of the Golgi complex and in close association with mitochondria in the mitochondrial rows separating the myofilament masses. These dense, single membrane-bounded bodies were approximately 0.1-0.4 μ in diameter, presumably represent the coalescence of a number of smaller Golgi vesicles and show acid phosphatase activity in their matrix. In some cases, (1) primary lysosomes had coalesced with double-membrane bounded residual bodies that were of the same size and shape as mitochondria but were not recognizable as double limiting membrane mitochondria because of the densification of their matrix and lack of recognizable cristae. In other instances, however, (2) images strongly suggested that primary lysosomes coalesced directly with mitochondria, since the partially degraded mitochondrial structure showed densification of the matrix but clearly discernible cristae and traces of double limiting membranes. In still other instances, (3) partially degraded mitochondria with densification of the matrix but clearly discernible

cristae and double limiting membranes were observed. These are similar to the second type mentioned except that primary lysosomes were out of the plane of section. Neither the solitary, partially degraded mitochondria nor those with which primary lysosomes had coalesced showed any indication of having been segregated by membranes of the sarcoplasmic reticulum, commonly observed in many other cell types. This direct coalescence of mitochondria with primary lysosomes without their prior segregation by cytomembranes from other components of the sarcoplasmic matrix represents a different and heretofore unreported mechanism of mitochondrial degradation resulting from lysosomal activity. Moreover, because of the lack of segregating membranes surrounding the mitochondria, it is highly likely that this wave of degradative activity spreads from one mitochondrion to others by their coalescence since many micrographs suggest that this has occurred. A second but relatively rare method of mitochondrial degradation in older myocardial cells was their segregation and partial digestion in autophagic vacuoles (secondary lysosomes). In addition to mitochondria, almost every autophagic vacuole contained large clusters of glycogen particles, existing as individual β particles segregated for their presumed degradation.

It is reasonably clear from the present studies that the degradation of mitochondria by lysosomal activity represents one major source of residual bodies in older rat myocardial cells. Although clusters of glycogen were segregated for their presumed lysis in autophagic vacuoles, the digestion of this endogenous energy reserve probably does not contribute to the cellular residue (residual bodies), since the simple sugars can be unloaded and reutilized.

Lipid droplets. A third major change in older myocardial cells was an apparent increase in the number of lipid droplets and an apparent change in the nature of the lipid, as exemplified by increases in density and decreases in solubility of the droplet contents. Even more interesting, however, was the observation that in older animals, lipid droplets were commonly segregated either partially or completely in autophagic vacuoles. Increased lipid in older cells could be explained by a shift in substrate utilization from relatively more endogenous lipid to more exogenous substrates than in the younger cells. The latter could result from different controls of intermediary metabolic pathways in older cells. The apparent change in nature of the lipid droplets with age suggests that the lipid in older myocardial cells is in a less readily available form, such as phospholipid. Biochemical evidence by Olsen and Hoeschen has shown that triglycerides and to a smaller extent diglycerides are the principle fatty acid esters metabolized in rat hearts and that phospholipid content remained constant. Even though the cardiac phospholipid pool is not stagnant, biochemically it has been shown that the turnover rate is much slower and less variable. Although the biochemical work of Olsen and Hoeschen and the electron radioautographic work of Stein and Stein lends support to the possibility that lipid in droplet form of the older rat myocardial cells may well be phospholipid, as suggested by the present studies which have shown increases in electron density, decreases in solubility and the presence of myelin caps on the lipid droplets, there is unfortunately no direct or indirect published information on changes in amount and nature of lipid in myocardial cells with age. The partially or completely segregated lipid droplets by smooth membranous elements of the sarcoplasmic reticulum in

older myocardial cells, for their degradation in autophagic vacuoles by either lysosomal activity or peroxidation or both, lends further morphological support to the possibility that the nature of the lipid has changed with age. Furthermore, many images interpreted to be a sequence of lipid droplet degradation to residual bodies clearly indicates that endogenous lipid reserves in old myocardial cells represent the second major observed cell component from which residual bodies arise.

It is clear from the present studies that residual bodies in the rat myocardial cells originate from the degradation of two major cell components: (1) the mitochondria and (2) endogenous energy reserves. The question then arises as to what causes residual bodies to accumulate in the older over the younger cells. Does the myocardial cell have the capacity to eliminate residue through its growth and developmental sequences or does it lose this capacity with age? Or does degradation or organelles occur at a greater rate than the cell can extrude the residue? In answer to these questions, the latter appears to be the case since there was no loss in the capacity of the cell to eliminate residue via the extensive T-system or at the peripheral cell surface. The question then arises as to what properties of the older myocardial cells might account for the induction of increases in lysosomal activity leading to the accumulation of residual bodies. Though these questions cannot be clearly answered from the present study, it is possible that the altered nature of the endogenous lipid, in droplet form and its unavailability for utilization might in themselves serve as feed back stimuli for the induction of autophagy and lipid droplet degradation by lysosomal and peroxidative activity. Even more interesting, however, is the highly specific, direct coalescence of mitochondria with primary lysosomes. This heretofore unreported process of degradation might suggest that mitochondrial membranes are altered in structure either due to errors during synthesis or by the gradual accumulation with time of unidentified factors which could also serve as feedback stimuli to induce lysosomal synthesis and increased lysosomal activity, a suggestion made by Brock and Hay in studies of chick fibroblasts. It is also possible that increased lysosomal synthesis and activity, leading to the accumulation of residual bodies with age, is genetically programmed. Although there is no definite evidence for these suggested possibilities, it is reasonably clear that the extent of degradation could be markedly important in altering the capacity of the heart to function normally with age.

A manuscript entitled "Ultrastructural Changes in the Rat Left Ventricular Myocardial Cells with Age" prepared during the past two months will be submitted for clearance and to the J. Ultrastructure Res. for publication.

Significance to Biomedical Research and the Program of the Institute: This is the first ultrastructural study to show changes in the rat myocardial cells throughout an age span of 6-33 months. The function of lysosomes and the ontogeny of residual bodies in myocardial cells are being established. This information is fundamental to the understanding of myocardial function with age.

Proposed Course of Project: Although we have fulfilled most of the proposed course of the project listed in the last annual report, further extended

electron histochemical studies will be carried out on the left ventricular myocardial cells in attempts to determine in greater detail mechanisms of lysosomal activity in myocardial cells with age. Moreover, it is anticipated that ultrastructural and electron histochemical studies of the papillary muscle of the left ventricle will be initiated since changes in these muscles not only reflect valvular function with age but are slender, contain fibers more consistently parallel than in any other part of the ventricle, thus enabling them to be easily stretched and oriented with ease. It is also hoped that it will be possible to carry out critically needed biochemical studies, correlated with the same region of the heart in which the marked ultrastructural changes have been observed to determine to what significant extent changes in lysosomal activity and thus degradation of the energy machinery to residual bodies in the myocardial cell might alter the oxidative capacity and thus the functional ability of the myocardium with age.

Honors and Awards: Dr. Travis was elected to the Council of the Washington Society for Electron Microscopy.

Dr. Travis was invited to speak at an NICHD Scientific Seminar sponsored by Dr. Charles Lowe.

Publications: None

Serial No.: HD-CCP1
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Morphology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Changes in the cells and extracellular matrices during growth, development and ageing

Previous Serial Number: Same

Principal Investigator: Dorothy F. Travis (40%)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	0.9
Professional:	0.4
Other:	0.5

Project Description:

Objectives: The broad objectives of this program are directed at understanding basic structural and functional principles underlying the activities of dividing, renewable cells as they are associated with synthesis, organization and changes in organization of extracellular matrices during growth and developmental sequences. Specifically, considerations will include little understood principles in both vertebrates and invertebrates which are related to (a) synthesis, organization, orientation, and mineralization of the structural components of the extracellular matrices and to (b) mineral metabolism including mechanisms of storage, release and resorption of inorganic components of skeletal structures.

A. The collagenous mineralized system of the sea urchin endoskeletal plate. Since the ultrastructural changes in regenerating sea urchin spine is now being studied by Dr. Heatfield, further concomitant work on the plate will not be resumed until sometime later this year.

B. The chitin-arthropodin non-mineralized and mineralized system of the crayfish gastrolith disc.

Background: As a brief background to this study, it is essential to indicate that the presence of a rigid calcified exoskeleton in this

group of animals (the arthropods) restricts their visible growth in size to well defined periods which are characterized by the animals shedding or molting of its old exoskeleton. In stages prior to this, beginning at 2 weeks preceding the molt when the animals do not feed, (Stage D_o--D_4) breakdown and resorption of both organic components and mineral salts occur in the old exoskeleton. These resorbed mineral salts from the old exoskeleton are deposited in some soft tissues (hepatopancreas) and some are stored in extracellular matrices concomitantly synthesized and deposited by the gastrolith epidermis, called the gastrolith, located in the anterior-lateral walls of the stomach. Moreover, when the animal sheds its old exoskeleton, the mineral salts from the gastrolith are resorbed and reutilized in calcifying the new exoskeleton (Stages A and early B) until feeding begins at 3 days to 1 week following the molt. These cyclic phenomena of resorption from the skeleton and deposition of mineral salts in soft tissues and the gastrolith resembles a number of ageing phenomena observed in mammals; that is, osteoporotic changes seen in the acetabulum and vertebrae with concomitant mineral deposits in the cartilagenous regions of the ribs and articular cartilagenous joints of long bones and other regions. This system uniquely provides the opportunity (1) for studying poorly understood mechanisms that are similarly operative in mammalian bone and cartilage during growth and developmental sequences and (2) to separate the activities of the epidermis in the gastrolith disc as it is associated with synthesis, orientation and organization of the chitin-arthropodin fibrils within a non-mineralized laminated extracellular matrix (Stage A--C) from its activities as it concomitantly synthesizes and releases both chitin-arthropodin and inorganic mineral components, orients, and organizes them in the calcifying matrix of the gastrolith (Stage D_o--D_4).

Limited Objectives: Since most of the background information cited pertains to light microscopic studies done by the present author a number of years ago, it is the purpose of the present study to determine what ultrastructural changes occur in the epidermal cells of the gastrolith disc as synthesis of the organic matrix of the gastrolith and mineral deposition ensue (Stages D_o--D_4). The present annual report is limited to very early stages of gastrolith deposition (Stage D_o).

Methods Employed. The fresh water crayfish, Orconectes virilis Hagen, trapped and maintained in the laboratory and of sizes ranging from 30--39 mm carapace length, were used in these studies. Under 0.1 M Sorensen's phosphate buffered 2.5% gluteraldehyde pH 7.4 at 4°C, the gastrolith discs with contained gastroliths were removed and fixed for a total of 48 hours. Tissues were then demineralized in 0.07 M EDTA in 0.43 M Tris made up in 10% formalin at 4°C for a period of one month. The tissues were throughly washed in 0.1 M Sorensen's phosphate buffered pH 7.4 at 4°C; post fixed in 0.1 M Sorensen's phosphate buffered 1% osmium at 4°C for a two hour period. Tissues were then prepared by standard procedures for electron microscopy.

Major Findings. The major findings reported here relate to EDTA demineralized material that was processed in the late fall and winter. After

sections were cut, studies were begun in February of this year and must be considered as a tentative statement of progress.

Structure and function of the epidermis: during early gastrolith deposition (Stage D_0): The individual cells are tall, narrow, columnar types with very contorted lateral cell membranes from mid-way of the cell height to the apical ends, accounting for a highly irregular branched like appearance of the apical ends of the cells. Large intercellular channels from the blood (hemolymph) sinuses of the connective tissue penetrate to within about 7 μ of the cell surface, enabling wandering blood cells to penetrate deeply into these channels and for solutes and solvents to be exchanged to and from the blood (hemolymph) and the epidermal cells. Within the apical half of the epidermal cells are many microtubules oriented with their long axis parallel to that of the cell. The presence of these microtubules in these regions of the cell suggests that the apical processes of the cell are highly contractile and that they may also function in internal movements of the cytoplasm and alterations in cell shape. Basally, the epidermal cells are limited by a basal lamina, which abuts either directly on blood sinuses or closely apposed cells of the connective tissue, cells of Leydig. The nucleus of the epidermal cells is located nearer the basal portion of the cell. It is characterized by the presence of some dense granular heterochromatin distributed irregularly throughout the nucleoplasm with some concentrations around the periphery of the nucleus. The less dense euchromatin is abundant, indicative of the fact that the cells are metabolically very active. A single nucleolus, somewhat peripherally located is evident within the nucleus. The epidermal cells are marked by the presence of an abundant rough surface endoplasmic reticulum with cisternae arranged in long and short parallel and branching arrays, indicative of an actively protein synthesizing cell. The most striking feature of this system is the presence of expanded cisternae which contain large well ordered protein crystals with 107A periodicities. These expanded cisternae with the enclosed stored protein crystals are found almost exclusively from the peripheral, mid-nuclear zones to its basal poles. There appear to be at least two rather small Golgi complexes, one at the basal nuclear pole and one peripheral to the apical nuclear pole. These consist of approximately three flattened saccules and many small Golgi vesicles of varying density. Mitochondria, roughly paralleling the long axis of the cell are more concentrated in apical regions of the cell with fewer being observed in the mid- and basal portions.

During the early stages of gastrolith deposition, the apical processes of the epidermal cells are rather blunt and irregularly shaped and are in contact with the developing gastrolith matrix. These processes contain many single, membrane-bounded, moderately electron dense, coated vesicles filled with fine granular material and coated with filamentous material on their surface. These vesicles closely resemble Golgi vesicles freed from the Golgi complex peripheral to the apical nuclear pole. The contents of these vesicles appear to be released into the developing gastrolith matrix by reversed pinocytosis. Since the Golgi apparatus is known to be involved in the synthesis of complex carbohydrates, it is possible that these coated vesicles contain either macromolecules of, or precursors to chitin and/or macromolecules of the protein, arthropodin. Only much further work in

electron histochemistry and radioautography can determine this. The gastrolith matrix in these early stages of formation consists almost entirely of granular and filamentous material, resembling that of the contents of the coated vesicles, and not showing any degree of orientation or organization, as is shown in preliminary studies of later stages of gastrolith formation. Another most conspicuous feature in the apical regions of the epidermal cells during early gastrolith formation is the presence of extremely large single membrane-bounded residual bodies. Although these bodies appear to be heavily concentrated in the apical regions of the cells, they are similarly found in the basal and middle zones of the cell. Their contents appear to consist of few remnants of, what are interpreted to be, mitochondrial membranes and vesicular material. These residual bodies represent massive regions of the cell and its components which have undergone degradation by lysosomal activity. Although the mechanisms of lysosomal activity and the ontogeny of the residual bodies in these cells are not known at this time, it would appear, from only preliminary ultrastructural studies of later stages in gastrolith deposition (Stage D_2--D_4) and previous correlative work done at light microscopic levels, that the function of lysosomes during this early stage of gastrolith deposition (when the animals have ceased to feed, when breakdown and resorption of both organic components and mineral salts are occurring in the old exoskeleton) is that of digesting cell components for the purpose of reutilizing these breakdown products in the synthesis of the gastrolith matrix. Especially important in this respect would be the reutilization of lipids (sterols and phospholipids) which constitute about 10% of the organic components of the gastrolith matrix (Travis, unpublished). The presence of large protein crystals in expanded cisternae of the endoplasmic reticulum during this early stage of gastrolith formation and their absence in other stages prior to molt, which we have examined on a preliminary basis (Stage D_2--D_4), suggests that either synthesis of proteins were stock piled prior to the beginning of gastrolith synthesis when the animals were feeding or that the amino acids involved in their synthesis come from breakdown and resorption of the old exoskeletal proteins prior to the molt. If the latter is so, the amino acids are recycled via the blood (hemolymph) to the epidermis for reutilization in gastrolith synthesis during these periods when the animals do not feed. Only further work can answer these questions.

Significance to Biomedical Research and the Program of the Institute: Now being established are structural and functional principles of the activities of cells which are synthesizing, organizing, orienting, and mineralizing the structural components of an extracellular matrix, the gastrolith. These functional principles intimately relate to similar mechanisms that are operative in developing vertebrate bone and cartilage. Changes in these dividing cells during periods of growth and development will lay the foundation for later studies of age associated changes in these same cells when such activities cease.

Proposed Course of the Project: Studies underway are planned to define further the ultrastructural and functional principles underlying the activities of the gastrolith disc epidermis as it is involved in the intermediate and late stages synthesis of the mineralized gastrolith (Stage D_2--D_4),

during periods when the animals do not feed. Electron histochemical studies are also planned to elucidate the mechanism of lysosomal activity, the ontogeny of the residual bodies and to more clearly define the function of lysosomes in these cells.

Honors and Awards: None

Publications: None

Serial No.: HD-CCP11

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Morphology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ultrastructural studies of the regenerating sea urchin spine during growth and developmental sequences

Previous Serial Number:

Principal Investigator: Barry M. Heatfield (100%)

Other Investigators: Dorothy F. Travis (20%)

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.2
Professional:	1.2
Other:	0.0

Project Description:

Objectives: The overall objectives of this project are to delineate and characterize, at the light and electron microscopic levels, the cells involved in the synthesis, organization, and mineralization of the structural components of the extracellular collagenous matrix of endoskeletal tissues of echinoderms, and to follow changes in these cells throughout developmental sequences. The present report is concerned with the preliminary results of studies on the morphology and ultrastructure of cells in the regenerating calcified dermis of fractured spines of the sea urchins, Strongylocentrotus purpuratus and Arbacia punctulata.

As background for the above objectives it should be pointed out that growth of the calcareous endoskeleton of echinoderms during developmental sequences has not been studied in detail. Previous work by Dr. Dorothy F. Travis of this laboratory has shown that primary stages of calcification are initiated by nucleation of young crystals of calcite in specific ordered regions of collagen fibrils of the organic matrix. However, during secondary stages of calcification, further ordered growth appears to occur by recrystallization phenomena in interfibrillar spaces and in other amorphous regions of the matrix. Ordered secondary stages of calcification suggest that cells of the matrix play a significant role in crystal growth. However, the cell-type or types responsible for synthesis of the collagenous matrix during

primary stages of calcification and ordered crystal growth during secondary stages of calcification have not been fully identified and characterized at either light or electron microscopic levels, nor has synthesis of the organic matrix been investigated.

Recent work (Heatfield, 1970, 1971a, 1971b) has shown that regenerating spines of sea urchins offer unique advantages as a model system for the study of calcification and associated processes in echinoderms during growth and developmental sequences. By simply cutting off distal portions of spines, calcification is initiated simultaneously in vivo or in vitro in all operated spines a few days after fracture as part of the regeneration process. Thus, it is possible to follow calcification and associated cellular activities from the very earliest to the very oldest stages of development and differentiation in many spines from the same or different animals and to study these under different environmental conditions. The preliminary results obtained with light and electron microscopy of this model system form the basis of the present report.

Methods Employed: Light microscopy: Primary interambulacral spines of the sea urchin, S. purpuratus, were fixed at various stages of regeneration in vivo and in vitro in neutral buffered 10% formalin in sea water at room temperature, then decalcified in 10% EDTA (pH 7.4) prior to dehydration and paraffin embedding. Conventional histochemical stains for carbohydrates, proteins, nucleic acids, and fats were applied to histological sections cut on an ordinary microtome. This material was brought from UCLA to GRC for analysis.

Electron microscopy: Regenerating spines of the sea urchins, S. purpuratus and A. punctulata, were fixed in a variety of cold, buffered solutions of glutaraldehyde followed by/or osmium tetroxide in combination with salts or sucrose to raise the osmolality close to that of sea water (1000 mOsm). Fixed tissues were dehydrated and processed for electron microscopy by standard procedures.

Major Findings: Previous work has shown that the spines of sea urchins consist basically of a fenestrated calcareous endoskeleton which is permeated throughout by a dermis of connective tissue containing migratory coelomocytes. The endoskeleton of the spine can be divided longitudinally into a proximal base and a distal tapered shaft. The outer surface of the endoskeleton is covered by an epidermis continuous with that of the body wall to which the base of the spine articulates via a ball-and-socket joint, being operated and secured by muscle and connective tissue. Following experimental fracture with scissors, healing of the wounded surface of the spine shaft begins, consisting of an inward migration of epidermis from the wound margin. This process is completed about two days after fracture. By the third day after fracture, calcification is initiated in the regenerating dermis of the spine. Complete regeneration of the lost portion of the spine shaft usually takes about sixty to ninety days.

Studies carried out to date on the histomorphology, histochemistry, and ultrastructure of cells occurring in the calcified dermis at the tip of regenerating spines reveal the existence of at least four different cell-types. All are apparently capable of migration since they occur in regenerated tissue throughout growth and developmental sequences.

Histology and histochemistry: As observed in the light microscope, three of the four cell-types contain various cytoplasmic inclusions. The characteristics of these cells are as follows: (1) "Eleocyte"--This cell-type may vary in profile, but is commonly spindle shaped and may be 35 μ or more in length. The round to ovoid nucleus is about 3 μ in diameter and is centrally located. The cytoplasm is filled with small granules ranging in diameter from about 0.4 to 0.6 μ. The histochemistry of these granules suggests they are composed largely of muco- or glycoprotein, or possibly a neutral mucopolysaccharide. They do not appear to be glycogen. These granules are frequently released to the surrounding tissues throughout the spine. This phenomena is particularly conspicuous adjacent to the fracture plane during early stages of regeneration, and granules characteristic of this cell-type are not evident in explants after regeneration for 2 to 3 days in vitro following experimental fracture. These results imply that there is a rapid turnover of these granules which appear to function as a nutritive substance for metabolism by spine tissues. (2) "Granulocyte"--This cell-type varies in profile, but is generally rounded to ovoid and ranges in diameter from 6 to 8 μ. The rounded to stellate nucleus is about 2.5 μ in diameter and may be slightly eccentric. The cytoplasm is filled with granules ranging in diameter from 0.8 to 1.2 μ, which are largely protein in composition. This cell-type is particularly evident in the epidermis, but does occur throughout the spine. No conspicuous changes take place in this cell-type during regeneration of spines in vitro. The function of this cell-type must await further investigation. (3) "Vacuolecyte"--This cell-type may vary in shape but is generally round to ovoid and ranges from about 9 to 12 μ. The round to stellate nucleus may be eccentric and is about 2.4 μ in diameter. The cytoplasm is filled with large opalescent vesicles which range from about 2 to 2.5 μ in diameter and contain acid mucopolysaccharides. No conspicuous changes take place in this cell-type during regeneration of spines in vitro, and its function is unknown. (4) "Fibroblast"--This fourth cell-type, the major cell-type present, is the connective tissue cell. Definitive cell boundaries are not discernible in the light microscope so that cell shape cannot be defined at this level. The ovoid nucleus is about 2.5 μ in diameter. The cytoplasm of these cells does not contain conspicuous granular material and shows no strong affinity for any particular histochemical stain. Its role in the synthesis and mineralization of the collagenous extracellular matrix remains to be established.

Ultrastructure: In the electron microscope, several cell-types are distinguishable, though representatives of all cell-types present may not have been observed to date, and, thus, it is not possible to conclusively establish identities between these cells and those described with the light microscope above. Cell type A--Only a few of these cells have been observed so far, and no nucleus has been evident. This cell is circular to ovoid in profile. The cytoplasm is filled with small vesicular bodies containing either amorphous or fibrous material. Fusion of adjacent bodies at some points of contact may occur. Small, apparently empty vacuoles also occur within the cytoplasm. Relatively few mitochondria are present and occasionally occur within the bodies described above. Little rough- or smooth-surfaced endoplasmic reticulum is evident. A few very dense, granular bodies are also present. This cell-type may correspond to the

"granulocyte" described above with the light microscope. Cell type B--This is the largest cell observed so far and may be ovoid to circular in profile. The nucleus is characterized by granular euchromatin surrounding patches of more electron dense heterochromatin. The cytoplasm contains membrane bound vesicles of variable size which contain finely granular material of less electron density than that contained in the bodies described in the previous cell-type. These vesicles are generally similar within a given cell and occasionally appear to have lost some or all of their contents, though the differences observed probably represent variations within a single cell-type. Few mitochondria and little rough- or smooth-surfaced endoplasmic reticulum has been observed in these cells. This cell-type may correspond to the "vacuolecyte" described above with the light microscope. Cell type C--This cell-type varies somewhat in shape being generally round to ovoid. The euchromatin of the nucleus is similar in density to the relatively scanty cytoplasm and surrounds a granular nucleolus. The heterochromatin is distributed principally around the periphery of the euchromatin except near the nuclear pores. The external surface of the nuclear membrane is studded with ribosomes. A few mitochondria are present and there appears to be at least two to three well-developed Golgi apparatuses consisting of about five parallel lamellae associated with small vesicles. The rough-surfaced endoplasmic reticulum, though scanty, is organized into a few flattened cisternae in the cytoplasmic matrix. Cell type D--This cell is characterized by numerous irregularly shaped pseudopodia containing mitochondria and rough-surfaced endoplasmic reticulum. The nucleus contains a single granular nucleolus embedded in a less dense nucleoplasm. A peripheral, patchy distribution of heterochromatin characterizes this cell-type. The cytoplasmic matrix contains a well-developed rough-surfaced endoplasmic reticulum which forms numerous narrow cisternae. At least two well-developed Golgi apparatuses consisting of approximately five lamellae are present in the cytoplasm.

The identity of cell-types C and D remains uncertain, though they may constitute what was previously described above with the light microscope as the connective tissue cell-type. Neither cell-type has the cytoplasmic granules nor bodies observed in eleocytes, granulocytes, or vacuolecytes. It would not be surprising if additional cell-types are found (i.e., eleocytes) as this work progresses. All of the cell-types described above occur in portions of the regenerating spine tip where active mineral deposition is taking place. However, the changes occurring in these cells during growth and developmental sequences and their precise role in the synthesis and mineralization of the extracellular collagenous matrix must await further investigation.

In addition to cells of the dermis, bundles of collagen fibrils with an axial periodicity of about 600 Å occur in the extracellular matrix together with numerous smaller fibrils which were frequently observed in close proximity to the mineral surface. However, the ultrastructure of these smaller fibrils has not yet been accurately defined.

Significance to Biomedical Research and the Program of the Institute:

Consistent with a comparative study of changes in cells and extracellular, chitin-arthropodin matrices of invertebrates or collagenous matrices of

both invertebrates and vertebrates at the ultrastructural level during growth and developmental sequences, the regenerating sea urchin spine offers unlimited potential for investigation of cellular and extracellular changes associated with synthesis and mineralization of collagenous matrices of the echinoderm endoskeleton. Because of the close similarity in structural organization between the echinoderm endoskeleton, vertebrate bone, and, in many respects, that of cartilage, it is felt that the principles underlying the activities of cells associated with synthesis and mineralization of the collagenous system of echinoderms during growth and developmental sequences are similarly operative in vertebrate bone and cartilage. Because regeneration of fractured spines is completed in a relatively short period as mentioned earlier, study of this experimental system offers the possibility of contributing greatly to our understanding of changes in the activities of cells with age.

Proposed Course of Project: It is essential that detailed descriptive work at the light and electron microscopic levels be continued and that a thorough delineation and characterization of the cells in the calcified matrix of the regenerating spine be completed prior to the initiation of studies on the respective roles of these cells during synthesis and mineralization of the extracellular collagenous matrix throughout growth and developmental sequences by the use of appropriate radioisotopic metabolic intermediates and radioautographic analyses at light and electron microscopic levels.

Honors and Awards: None

Publications:

Heatfield, B. M.: Calcification in echinoderms: effects of temperature and Diamox on incorporation of calcium-45 *in vitro* by regenerating spines of *Strongylocentrotus purpuratus*. *Biol. Bull.* 139: 151-163, 1970.

Heatfield, B. M.: Growth of the calcareous skeleton during regeneration of spines of the sea urchin, *Strongylocentrotus purpuratus* (Stimpson): a light and scanning electron microscopic study. *J. Morph.* In press.

Heatfield, B. M.: Origin of calcified tissue in regenerating spines of the sea urchin, *Strongylocentrotus purpuratus* (Stimpson): a quantitative radioautographic study with tritiated thymidine. *J. Exp. Zool.* In press.

Serial No.: HD-CCP5
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Age changes in cellular populations of the nervous system

Previous Serial Number: Same

Principal Investigator: Charles H. Barrows (25%)

Other Investigators: Melvin H. Epstein

Cooperating Units: The Johns Hopkins Hospital
Baltimore City Hospitals

Man Years:

Total:	1.75
Professional:	0.25
Other:	1.50

Project Description:

Objectives: By exploring certain selected biochemical systems in individual cell populations it is hoped that some aspects of the aging of the mammalian nervous system will be illucidated. It is also planned to explore parallel methods in samples of living human brain.

Methods Employed: A modified Cartesian diver was used for studying O_2 uptake in glucose and endogenous O_2 uptake in saline.

The ultra micro trapping method previously developed was used to measure Dopa-decarboxylase and glutamic acid decarboxylase.

Major Findings: The studies in relation to animal age are now completed. It appears that Dopa-decarboxylase does not change with age in either the cerebellar, molecular layer, granular layer or white matter. No changes are found for frontal lobe granular, molecular or white matter. Small declines may occur in the peripheral ganglia and the peripheral nerves. Several areas in the brain stem show no significant changes.

Glutamic acid decarboxylase activity was unchanged with age in the granular layer and molecular layer of the cerebellum and in granular, molecular and white matter of the cerebral cortex. No changes were found in several brain stem nucleic or spinal cord nuclei.

O_2 uptake was studied in the presence of glucose and in the absence of glucose in the aforementioned micro anatomical areas of the nervous system. No marked changes with age were apparent.

Significance to Bio-Medical Research and the Program of the Institute: The obvious major health problem of a large population of senile individuals will be even more acute when medical services are delivered to a greater proportion of our citizens. A basic understanding of senile brain function is essential to any therapeutic or preventative measures on clinical level.

Proposed Course of Project: This project has been discontinued.

Honors and Awards: Dr. Charles H. Barrows, Jr. was the recipient of the Robert W. Kleemeier Award and Lectureship in 1970 presented by the Gerontological Society for outstanding and meritorious contributions to research in the field of aging.

Publications:

Beauchene, R. E., Roeder, L. M., and Barrows, C. H., Jr.: The interrelationships of age, tissue protein synthesis, and proteinuria. J. Geront. 25: 359-363, 1970.

Wolfe, S., Cage, G., Epstein, M., Tice, L., Miller, H., and Gordon, R. S., Jr.: Metabolic studies of isolated human eccrine sweat glands. J. Clin. Invest. 49: 1880-1884, 1970.

Strehler, B. L., and Barrows, C. H., Jr.: Senescence: cell biological aspects of aging. In Schjeide, O. A. and de Vellis, J. (Eds.): Cell Differentiation. Princeton, N. J., D. van Nostrand Co., Inc., 1970, pp. 266-280.

Barrows, C. H., Jr.: The challenge--mechanisms of biological aging. Gerontologist 11: (1), (Part I), 5-11, 1971.

Epstein, M. H.: Studies on the relative susceptibility of cortical elements to mechanical trauma. J. Neurosurg. In press.

Serial No.: HD-CCP3 (c)

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A multi-disciplinary investigation of progeria (Hutchinson-Gilford syndrome), a disease of premature aging in children

Previous Serial Number: Same

Principal Investigator: W. Reichel

Other Investigator: M. Sussman
G. Becker

Cooperating Units: Baltimore City Hospitals
Carnegie Institute of Washington
Goucher College

Project Description: This project has been discontinued.

Publications: None

Serial No.: HD-CCP10

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Alteration of normal developmental sequences in chick embryo by cycloheximide administration

Previous Serial Number: Same

Principal Investigator: W. Reichel

Other Investigator: C. Barrows

Cooperating Units: None

Project Description: This project has been discontinued.

Publications: None

Serial No.: HD-CCP2

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Lipofuscin pigment accumulation as a function of age and localization in animal tissues, and its pathophysiological significance

Previous Serial Number: Same

Principal Investigator: W. Reichel

Other Investigator: M. Epstein

Cooperating Units: None

Project Description: This project has been discontinued.

Publications: None

Serial No.: HD-AG13

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Aging in the rotifer

Previous Serial Number: Same

Principal Investigators: N. Meadow (100%)

Other Investigators: C. Barrows (25%)

Cooperating Units: None

Man Years:

Total:	3.25
Professional:	1.25
Other:	2.00

Project Description:

Objectives: The rotifer has many characteristics which make it an excellent organism to serve as a model system to study biological aging. Among these are a short lifespan, parthenogenesis and poikilothermia. In addition it has been reported that nutrition and parental age influence the lifespan of this organism. The general purpose of this program is to determine the physiological, biochemical and histological characteristics of rotifers at various stages of their lifespan and to determine the basic biological mechanisms responsible for senescence and for those factors which influence the longevity of these organisms.

Methods Employed: Specialized techniques for aseptic rotifer culture; and specialized histological techniques for obtaining sections from a few, or even single rotifers. In addition, microchemical, radioisotope assay and biochemical techniques are used.

Major Findings: (1) Histochemical studies were initiated to examine the nature of Inclusion Bodies, cellular organelles which were found to increase greatly in concentration in several organs of older rotifers. Earlier histochemical studies have shown that the age-dependent distribution of two lysosomal enzymes, acid phosphatase and β-glucuronidase, was consistent with the age-dependent alterations in Inclusion Body concentration. A histochemical study of a third lysosomal enzyme, E600

resistant esterase, has been carried out. The activity of this enzyme was found to increase in the stomach, ovaries and dorsal gastric gland of older rotifers, a pattern which is also consistent with the age-dependent changes in Inclusion Body concentration. Thus the histochemical studies strongly suggest that Inclusion Bodies are some form of lysosome.

(2) Since the studies described above have implicated lysosomes in the aging process in rotifers, an attempt was made to alter lifespan by growing the animals in the presence of substances known to alter the stability of lysosomal membranes. To date, the effects of hydrocortisone and chloroquine, both stabilizers of lysosomal membranes, have been tested. Chloroquine was found to increase longevity, primarily among very old rotifers. This substance doubled the further life expectancy of rotifers at the age of 50% survivorship. Old animals treated with chloroquine were much more active than their untreated controls. Hydrocortisone has no effect on longevity.

(3) Earlier studies of the effects of temperature and nutrition on longevity, and of age-dependent morphological changes, have shown that an event associated with the cessation of reproduction acts as a stimulus for senescent changes to occur. The stimulus may be either a release from an inhibitory effect of oocytes and maturing eggs, or an inductive effect of autophagia which occurs in the ovaries after the last oocyte is matured. Alteration of the duration of the reproductive period by killing ovarian nurse cell nuclei with a microbeam would enable us to specify which of the events is the critical one. Preliminary studies have been carried out which have shown that of the two available microbeam techniques, ultra-violet and laser, the UV beam is preferable. These studies have also shown that the reproductive rate can be reduced to at least half the normal rate, but that the treated animals are normal in other respects.

Proposed Course of Project: (1) The possible relationship between lysosomes and senescence in rotifers requires further investigation. At present assays are being set up to investigate whether there is an age-related increase in latent acid hydrolase activity, since latency is a specific property of lysosomal enzymes. Because several acid hydrolases which are found in lysosomes are also present in isozymic forms, techniques are being set up to study age changes in isozyme patterns using disc gel electrophoresis.

(2) The UV microbeam studies will be carried to completion.

(3) Earlier studies of changes in various enzymatic activities and ultrastructural changes in ribosomal distribution have suggested that there are age-dependent changes in the pattern of protein synthesis. In addition, several current hypotheses of aging predict age-dependent changes in protein complement. Therefore, a study has been initiated in collaboration with Dr. Sri Ram of the Laboratory of Pathology, NIAMD, which will attempt to detect changes in protein complement in old rotifers using immunological techniques.

Honors and Awards: Dr. Meadow was invited to lecture on the Biology of Aging at the Biology Division of the University of Maryland, Baltimore County, December 1970.

Dr. Meadow was invited to present a seminar at Richmond College of the City University of New York, February 1971.

Publications: Herold, R., and Meadow, N. D.: Age related changes in ultrastructure and histochemistry of tissues of rotiferan organs. J. Ultrastructure Res. 33: 203-218, 1970.

Meadow, N. D., and Barrows, C. H., Jr.: Studies on aging in a bdelloid rotifer. I. The effect of various culture systems on longevity and fecundity. J. Exp. Zool. 176: 303-313, 1971.

Meadow, N. D., and Barrows, C. H., Jr.: Studies on aging in a bdelloid rotifer. II. The effects of various environmental conditions and maternal age on longevity and fecundity. J. Geront. In press.

Serial No.: HD-AG37

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the comparative physiology of aging and environmental effects on the ageing process

Previous Serial Number: Same

Principal Investigator: M. A. Brock (100% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description:

Objectives: The broad objectives of this program are directed at understanding the fundamental structural and functional principles underlying the activities of cells as they may be modified by temperature during the ageing process. In poikilothermic species, the rate of ageing is generally thought to depend on ambient temperature; while in homoiothermic animals, it is believed, without substantiation, that hibernation with its characteristic drop in body temperature, can serve to extend life. Therefore, representative poikilothermic and homoiothermic species, a coelenterate (Campanularia flexuosa) and golden hamsters (Mesocricetus auratus), are being used to explore the effects of temperature on cellular ageing.

Methods Employed: Campanularia flexuosa colonies were cultured in aerated, artificial sea water at 4°, 10°, 17° and 24° for ageing studies and other determinations at each of these temperatures. The methods used for mapping the colonies and recording data relating to the development and longevity of the hydranths were reported in the Annual Report for 1970. Golden hamsters (Mesocricetus auratus) were individually housed at 24° and in a specially designed hibernaculum at 5°. Biochemical procedures, some developed for use in these laboratories, include enzyme activity measurements, column and thin layer chromatography, subcellular fractionation and cellulose acetate strip electrophoresis.

Major Findings:

A. Studies of environmental effects on Campanularia. The existence of persistent circannual rhythms (cycles of about a year's duration) has been confirmed through the extension of the experiments reported last year for a period spanning more than two annual cycles. The rhythms characterized so far include annual changes in hydranth longevity, upright longevity, growth habit, and responses to changes in ambient temperature; and annual periods of severe depression of the colonies and of gonangia development. It should be emphasized that these rhythms persisted despite constant environmental conditions. However, since there has been an assumed dependence of the longevity of poikilotherms on ambient temperature, in some of the following sections, the results will be related to the annual changes in sea water temperature at Woods Hole, Mass., the native environment of the Campanularia colonies used for these studies. The monthly averages of sea water temperature were calculated from U. S. Coast and Geodetic Survey daily records made at Woods Hole, Mass. over three years, 1967-1969. The highest temperatures (20.6-22.3°C) are reached in August, and they fall to their lowest levels (-0.76-2.74°C) in January and February.

(1) Hydranth longevity. In colonies cultured at 10° over the duration of the experiments, the mean life span (MLS) of hydranths during Winter months was approximately double that during the Summer months. Over three winters, the longest lived hydranths were observed during December 1968-January 1969 (MLS = 16.7 days); October-November 1969 (MLS = 15.7 days) and February-March 1970 (MLS = 16.6 days); and October-November 1970 (MLS - 15.6 days). Current data appear to correspond to that of February-March 1970. The Summer decline in longevity reached its lowest point in June 1969 (MLS = 7.4 days) and August 1970 (MLS = 9.7 days).

Similarly, cyclic changes in hydranth longevity were observed in the colonies cultured at 17° and 24°, with an approximately two-fold seasonal change in the mean life span. The longest lived hydranths at both temperatures lived during the months of November and April-May; the shortest-lived were observed during June-July.

At all of the culture temperatures, a decline in longevity occurred in mid-Winter. At 10°, the MLS dropped to 9.7 days in January-February 1970 and to 9.9 days in January-February 1971. In both years, the longest-lived individuals were observed just prior to and after this period. Interestingly, the Woods Hole Water Temperature reaches its yearly low at this time. Since it seemed reasonable to expect that the colonies might be adapted to environmental temperatures close to 0° during these months and that the 10° ambient temperature was high enough to provide a stressful situation resulting in a life-shortening effect, some colonies were transferred to 4°. The hydranths in these colonies lived for 15.3 days (MLS) in contrast to those at 10° which lived for 9.9 days (MLS). Furthermore, normal developmental sequences were observed at 4° during January-February 1971. This is in disagreement with Crowell's observation that development did not take place at 6°; however, it is an easily resolvable discrepancy if Crowell's data were taken from colonies during Summer months when the hydranths are likely adapted to higher ambient temperatures.

The cyclic changes in hydranth longevity can be correlated with the annual fluctuations in water temperature at Woods Hole. The shortest life spans in each constant environment were recorded during the months when the water temperature at Woods Hole is the highest; conversely, the longest life spans were seen during the months when the Woods Hole water temperatures are the lowest, with the exception of the life-shortening effect observed during January-February that was discussed previously. The implication is that the endogenous rhythms which presumably evolved in the natural environment partially as a result of selection pressures and which persist under constant environmental conditions in the laboratory, suggests that marked intrinsic cellular changes in the rate of development and ageing occur.

(2) Upright longevity. The marked changes in the growth and survival of uprights at 24° that were observed during January-February 1969 through 1970 were noted again in 1971. These changes were discussed in the 1970 Annual Report and will not be detailed here.

(3) The effects of transfer to different ambient temperatures. The periodic switches of colonies to warmer or colder environments that were described in the 1970 Annual Report were repeated during 1971 and confirmed the prior observations. In partial summary, hydranths transferred to a colder environment (17° to 10°) during the Winter months showed longer life spans the day of the transfer. In the same kind of switch during Summer months, there was a lag period before the hydranths became longer lived. This data and that of Section 3 are compatible with the hypothesis that there are seasonal adaptive changes in tolerance to environmental temperature: the "Winter" and "Summer" hydranths showing quite different responses to switches to cold.

(4) Severe depression of the colonies. A rather precisely timed annual depression in colony growth occurred in mid-Winter in 1969, 1970 and 1971. This period of diminishing growth and lack of replacement of hydranths corresponds to the time when the Woods Hole water temperature is at its lowest, and it terminates the period of decreased longevity of the hydranths and uprights in mid-Winter. In severe form, it can result in the death of the colonies. In less severe form, the uprights appeared devoid of cytoplasm and would be considered dead, except that after a lag time of 7 to 10 days new growth was initiated. Within a week, the colonies looked normal and growth was luxuriant. This striking event was observed in colonies at all temperatures; it was not prevented in the colonies switched to 4°. The precise nature of the timing of this event is evident from the dates of the end-point of the depression: January 30, 1969, February 20-March 4, 1970, and March 2, 1971.

B. Biochemical Measurements on Campanularia. Since previous ultrastructural studies have shown increased hydrolytic enzymatic activity reflected in the lysosomal degradation of cellular organelles in certain cell types with age, biochemical studies were undertaken to quantify these changes. The total acid phosphatase activity, measured with the substrate, β-glycerophosphate, doubled with age in hydranths at 10°. Similarly, the free activity which represents that of the secondary lysosomal enzymes doubled with age. Both increases in activity were statistically highly significant.

These results differ from previous determinations in hydranths at 17° which showed a rise in free or secondary lysosomal activity with age but no significant change in the total activity. When the total and free activity in hydranths either at 10° or 17° was expressed per unit protein or DNA, the increases were obscured due to small rises in protein and DNA concentration with age. This is undoubtedly a dilution effect and may be explained by the continued cell division and differentiation throughout the life of Campanularia hydranths that is directed primarily toward the replacement of intact nematocytes. Mitotic figures and stages in nematocyte differentiation were visualized ultrastructurally in previous work. Nematocytes with empty capsules from which the nematocysts have been expelled were observed to remain in the tentacular ectoderm. Thus, the cell types in which marked evidence of lysosomal activity with age was visualized ultrastructurally, the epitheliomuscular, neurosecretory, digestive and tentacular gastrodermal, are continuously being diluted with newly differentiated nematocytes.

The possibility that different isoenzymes of acid phosphatase are present in Campanularia colonies cultured at 10° and 17° and at different seasons of the year was raised by the results of experiments to determine the optimal hydrogen ion concentration for the enzyme assay. During the Summer, maximal total activity in hydranths from colonies cultured at 10° was observed at pH 4.2, whereas two peaks of activity, one at pH 4.2 and a less conspicuous one at pH 5.4, were seen in hydranths from 17° colonies. In the Autumn, there were shifts in both, with maximal activity at different hydrogen ion concentrations. The latent enzymatic activity presumably represents activity of enzymes bound in primary lysosomes not ruptured during homogenization and is calculated as the difference between the total and free activity. In hydranths from colonies cultured at 10° and 17°, maximal latent activity was observed at pH 4.6 (with a minor peak at pH 5.4) and at pH 4.2, respectively during the Summer. In the Autumn, the peaks of activity, like that of the total activity, shifted in the colonies cultured at both temperatures.

The above data suggested that a more refined electrophoretic analysis of the isoenzymes of acid phosphatase might reveal different banding patterns that could vary not only with the ambient temperature of the colonies and the pH of the incubation medium, but also with the stage of development at any given temperature and with the seasonal fluctuations in longevity. The present results span two midwinter months. In general, there is an increase in the number of bands, from 0 to 3, and in their intensity during the embryonic development of hydranths cultured at 10°. These changes were observed over the pH range 3.4 to 5.8 using the substrate, β-glycerophosphate. Preliminary data from a late developmental stage of hydranths from colonies at 17° showed fewer bands than were seen in the comparable stage from colonies at 10°. A most interesting change was observed in early March at the time the colonies were severely depressed and the hydranths were very short-lived. During this period, many of the developing hydranths never reach adulthood, but rather appear to undergo a modified form of regression or disintegrate. In the late developmental stages from colonies at 10° at this time, there were up to four bands that were of greater intensity than those observed previously. The values describing the intensity were, in fact, as high as

those recorded for regressing hydranths, and their pH optima were more similar to regressing hydranths than to adults. In adult hydranths as compared to those in advanced stages of development at 10°, there was generally increased intensity of comparable bands except at pH 4.6. At this pH, there were clearly differences in the reactivity of bands 1 and 4. Preliminary data from adults in colonies at 17° showed banding patterns similar to those from hydranths at 10° except for decreased intensity. There were marked changes during regression both in the banding patterns and in the number and intensity of the bands, with up to 5 bands at pH 5.0 and 5.8. In addition, there were obvious changes in the pH optima of the Band 1 and Band 4 enzymes. In summary, changes in the number and activity of isoenzymes of acid phosphatase present throughout development, adult life and regression of hydranths at 10° during midwinter were observed. Preliminary data suggest that the enzyme patterns in hydranths at 17° are somewhat different. Interestingly, at the time of severe depression of the colony, the late developmental stages resemble regressing hydranths more than adults.

C. The Effect of Hibernation on Longevity and on Age-Related Cellular Changes. In these experiments, since the life spans of control hamsters are to be compared with those that have hibernated, it is essential that the precise length or percentage of time spent in hibernation is known. Previous hibernation studies have depended on recording from implanted thermocouples or thermistors to determine body temperature. This procedure not only involves disturbing surgical techniques required to implant the thermistors but also the risk that the leads will remain in place for the duration of the experiment. Since golden hamsters are notoriously adept at dislodging leads of all kinds, a remote temperature monitoring system is being developed. Essentially, this consists of individual sensors, infra-red sensitive, positioned over each cage with leads to a multichannel scanner and recorder. The feasibility of this system has been demonstrated with one of these specially fabricated sensors. Currently, a battery of sensors is being supplied to test the monitoring system with a group of cold exposed and hibernating hamsters.

Among the indices of age that change most markedly in the Bezanson strain of golden hamsters is a yellow-brown, non-fluorescent pigment which is extractable from the liver and is chemically unlike lipofuscin. The concentration of this pigment increased 200-fold with age in the Bezanson strain. Our experiments have shown that the pigment also can be separated with and isolated from the liver nuclear fraction of the Albino Farms strain of golden hamsters currently in use. The pigment displays the published chemical characteristics, and its concentration in 8 month old hamsters falls in the range of that described for the Bezanson strain. It, therefore, should be extremely useful for comparisons of the rate of its deposition in liver cells of control and hibernating hamsters.

In previous studies using ion exchange column chromatography to quantify the pool sizes of glycolytic intermediates in erythrocytes, an unknown nucleotide was isolated from the red cells of control, cold-exposed and hibernating hamsters. Based on total phosphate, the concentrations of this nucleotide rose during hibernation. Its spectral characteristics are similar to inosine phosphates, however it does not elute in the fractions

that contained IMP, IDP and ITP in a separate column assay. Thin layer chromatography of this nucleotide, isolated from frozen erythrocyte extracts and also from extracts of freshly drawn blood, has recently confirmed that the material is not IMP, IDP or ITP; further tests to identify this material are in progress. The presence of an inosine-like compound in hamster erythrocytes is important in view of the role of inosine and adenosine as supplements to blood preservatives in extending erythrocyte viability in vitro. Similarly, the life span of erythrocytes during hibernation, in vivo, is markedly extended over non-hibernating controls.

Significance to Biomedical Research and the Program of the Institute: Manipulation of animals' life spans by changes in body temperature affords the opportunity to study basic regulatory mechanisms of ageing at the cellular level.

Proposed Course of Project: In the poikilotherm, Campanularia, the description of the persistent circannual rhythms is essentially completed except for more detailed analysis of some portions of the data. Investigations of the mechanisms of the endogenous cyclic changes in longevity in addition to those changes in longevity induced by temperature switches at the cellular level will be continued using correlative biochemical and ultrastructural techniques. The effects of hibernation of a homoiotherm, the golden hamster, on the longevity and on other physiological and biochemical phenomena will be explored using the procedures described in addition to other correlative biochemical and ultrastructural techniques.

Honors and Awards: Dr. Brock was elected Secretary of the Society for Cryobiology.

Dr. Brock was an invited chairman of a Metabolism Session at the Hibernation-Hypothermia IV International Symposium, January 1971.

Dr. Brock was invited to present two lectures, in invertebrate zoology and in developmental biology, at Goucher College.

Publications:

Brock, M. A.: Ultrastructural studies on the life cycle of a short-lived metazoan, Campanularia flexuosa. II. Structure of the old adult. J. Ultrastructure Res. 32: 118-141, 1970.

Brock, M. A.: The effect of ambient temperature on ageing and on lysosomal activity in Campanularia flexuosa. J. Cryobiology. In press.

Serial No.: HD-AG31 (8)

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Age-associated fine structural changes in cultured chick fibroblasts

Previous Serial Number: Same

Principal Investigator: M. A. Brock

Other Investigators: R. J. Hay

Cooperating Units: Carnegie Institution of Washington, Embryology Department

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

This project has been discontinued.

Publications: Brock, M. A., and Hay, R. J.: Comparative ultrastructure of cultured chick fibroblasts at early and late stages during their growth span. Carnegie Institution of Washington Yearbook 69: 600-601, 1971.

Brock, M. A., and Hay, R. J.: Comparative ultrastructure of chick fibroblasts in vitro at early and late stages during their growth span. J. Ultrastructure Res. In press.

Serial No.: HD-CCP9

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Age-related changes in the Daphnia carapace

Previous Serial Number: Same

Principal Investigator: David Hirschfeld

Other Investigator: Dorothy F. Travis

Cooperating Unit: Baltimore City Hospitals

Project Description: This project has been discontinued.

Publications: None

NICHD Annual Report
July 1, 1970 through June 30, 1971
Gerontology Research Center
Laboratory of Behavioral Sciences

"It was the best of times, it was the worst of times,... it was the spring of hope, it was the winter of despair."

Those remarkably insightful words, written more than 100 years ago to describe a time 70 years earlier, aptly summarize some aspects of the past year for this laboratory. Early in the year two key technicians resigned, and for four months, from October through January, the laboratory was without the services of a secretary. All of these positions have been filled now, however, the transitions have made the professional staff deeply aware of the importance of the jobs these employees perform. In addition to these specific personnel changes, the continuing uncertainty of personnel and budgetary support--in the three years of its existence, this laboratory has never seen more than a six month, non-emergency, operating budget--has had a pervasive, depressing effect. However, it is spring again.

The section of Human Learning and Problem Solving now has all the experiments on concept problem solving on-line in the Gerontology Research Center computer, i.e., the computer determines the information feedback which the subject receives, and catalogs each subject's responses for subsequent analyses. The next projected steps in this development are to: a) increase the range and complexity of problems being studied; and b) enable the subject to interact directly with the computer thereby reducing the personnel time.

Data from the Gerontology Research Center longitudinal study program show that problem solving ability declined significantly among men who were tested initially after the age of 60 and retested six to eight years later. This effect is not present among subjects who were under the age of 60 for their first test. The evidence indicates that the decline among the older men resulted from an inability to make logical inferences from already available information. This experimental measure of problem solving performance is probably assessing cognitive functions which are different from those measured psychometric tests since previous longitudinal studies which have used psychometric measures of cognition have reported no age-related changes.

Other experiments in this section have confirmed previous reports that information presented simultaneously to the two ears is recalled in ear order by many subjects, e.g., when four pairs of digits are presented to the two ears simultaneously, many subjects report all the digits presented to one ear and then all the digits presented to the other ear. Our experiments have carried these findings further by showing than any given subject shows a consistent tendency to report in one of two ways: virtually never in ear order and predominantly in ear order. Why some subjects should mix information from the two ears while others keep the data separate, and why a subject consistently falls into one class or the other, are questions for subsequent research.

Studies of the ability of patients with cardiac disease to control selected aspects of their cardiac function, and studies of the mechanisms by which monkeys control their heart rates are continuing in the Psychophysiology Section. Results from experiments with five patients with atrial fibrillation indicate that these patients can consistently, differentially control their heart rates, i.e., the patients can voluntarily and reliably speed and slow their hearts. These data should help resolve a controversy which has been in the cardiological literature ever since Sir Thomas Lewis concluded that ventricular rate in atrial fibrillation is a random event. Apparently it is not. More importantly, however, these data suggest that it is possible to significantly modify some functions of the atrio-ventricular node through volitional action.

Ongoing experiments with monkeys are designed to assess the relationship between state of arousal, and direction of control of pulse rate. In these studies each animal is trained to speed its heart and slow its heart. At the same time that the animal is learning heart rate control, or performing at at high level of learning, the percentage time during which it is producing high voltage, synchronized brain waves is also being recorded. This brain wave activity has been associated with a quiet, awake, relaxed state by previous investigators, whereas its absence in an awake subject has been associated with alertness and/or behavioral arousal. Very preliminary findings to date suggest that voluntary cardiac slowing is associated with a relatively "relaxed" brain while voluntary cardiac speeding is associated with a more "aroused" brain. These differences in brain wave activity occur in the absence of any apparent differences in motor activity; i.e., if one observes an animal while it is speeding and slowing its heart, he can not see any overt somatic differences between these two conditions.

Studies with two inbred strains of mice (C57BL/6J, long-lived, and A/J, short-lived) and F_1 hybrids showed that: a) longevity was overdominant, i.e., F_1 animals lived longer than either parental strain; b) both long-lived groups maintained a high level of activity throughout most of their life spans, whereas the short-lived animals declined in vigor disproportionately early in life; c) in all three groups, animals which were fed isocaloric, low protein diets (4% casein) early in life had significantly higher drive levels at maturity then did normally fed, control animals.

In other studies done in colloraboration with the section on Intermediary Metabolism, Laboratory of Biomedical Sciences, it was shown that infant rats, injected with p-chlorophenylalanine and phenylalanine, showed maze learning deficits when compared with saline injected controls. Other tests with these same groups showed that the experimentally injected animals also tended to be more active and were significantly less emotional.

In addition to the in-house research program summarized above, the laboratory initiated a contract with Dr. M. A. Wenger (Department of Psychology, University of California at Los Angeles) to carry out a follow-up study of about 2000 Army Air Force cadets whom he had tested 25 years earlier. The original tests included a battery of physiological and psychological measurements. The follow-up is designed to determine the incidence of

various diseases in the population, and how well the earlier test battery predicts these diseases. The basis for the contract is to determine whether the Wenger test battery has sufficient predictive validity to warrant its inclusion in the longitudinal study program at the Gerontology Research Center. This study is now at the stage of mailing questionnaires. To date 301 questionnaires have been returned completed. The major delay came from the fact that Wenger did not receive final Bureau of the Budget clearance on his questionnaire until three months after the request for final clearance was submitted.

Serial No. HD-AG2 (8)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Operant Conditioning of Heart Rate in Monkeys.

Previous Serial Number: Same.

Principal Investigator: Bernard T. Engel (50% of time).

Other Investigators: None.

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	2.10
Professional:	.80
Other:	1.30

Project Description:

Objectives: To train monkeys to voluntarily control their cardiac functions and to use this preparation to identify the physiological mechanisms associated with this control.

Methods Employed: These experiments are done with rhesus monkeys. The procedure involves several stages. First, a cannula is implanted into an external iliac artery under sterile surgical conditions. Following the surgery it is necessary to infuse 1.5 U/ml, heparinized saline into the catheter to keep the artery/cannula patent. This preparation permits adequate monitoring of blood pressure and pulse rate for at least six months.

Other surgical procedures depend on the specific experiments currently under way. In the experiments reported this year, stainless steel electrodes are chronically implanted in the parietal or occipital cortex of the brain. The purpose of these electrodes is to monitor non-specific electroencephalographic (EEG) activity of the brain in the 8-14 Hz range, and to relate changes in brain function to learned changes in heart rate. Previous research in other laboratories has shown that under conditions comparable to those in our experiments, these EEG changes can be used as indices of arousal of the brain. In general, the presence of EEG activity is associated with a quiet state whereas its absence is associated with an aroused state.

After the animal has recovered from surgery, heart rate training is begun. Each training session comprises about 75 minutes. During the first 25 minutes the animal is allowed to remain quietly in the sound-proofed booth with the door shut. During the last 10 minutes of this period automatic data processing equipment counts the animal's pulse, and displays its heart rate on a counter. At the same time, blood pressure, EEG activity, and control signals are recorded on analog magnetic tape. Average systolic and diastolic pressures and percentage time high voltage is present in the EEG are also displayed on counters every 128 sec. After the animal's baseline heart rate has been determined, control modules are set so that heart periods longer or shorter than the average basal rate can be automatically detected.

During the final 35 minutes of each session, the animal is provided with a signal from one of two differently colored lights. These are cue lights one of which it must associate with heart rate speeding, and the other of which it must associate with heart rate slowing. As long as the animal performs correctly the appropriate cue light will burn steadily; as soon as it begins to perform incorrectly, the cue light will flicker at one/second; during the period when the cue light is flickering, the animal will receive a 10ma, electric shock to its tail for .45 seconds at the rate of one shock/eight seconds. In essence then, the monkey must learn to keep the appropriate cue light burning steadily to avoid a shock by controlling its heart rate; it must either slow or speed its heart depending upon which cue light is functional.

Major Findings: This study will include four animals when it is completed so far one animal has been completely tested, two are virtually completely tested and testing of the fourth will begin shortly.

Analyses of the three animals tested to date are complete for the early acquisition phase of the experiment. Analyses for the later,stable stage of performance are not done.

Each of the three animals correctly controlled its heart rate. During the slowing sessions heart rate was decreased an average of about seven beats/min, systolic and diastolic pressure did not change, and the percentage time during which high voltage appeared in the EEG increased by four percent from baseline levels. During speeding sessions heart rate increased an average of about 16 beats/min, systolic pressure and diastolic pressure each increased about five mm Hg and the percentage time during which high voltage was present in the EEG decreased about three percent.

In addition to the measurements cited above, ratings of behavior were also made throughout each session. These results indicate very few behavioral differences between slowing and speeding sessions and only slight differences between animals (ratings are available only for two of the three animals).

There are frequent eye movements throughout all training sessions and there are a significant number of head movements as well. There are

almost no body movements. Each animal kept its eyes open throughout most sessions, and each tended to orient toward the cue light about half of the time. Thus, these data indicate few behavioral differences to relate to the physiological changes cited above. However, it must be emphasized that these results are limited to the acquisitive phase of the learning situtation, and they must be considered tentative. It is quite possible that results from later stages of the study,when the animals are overtrained, will reveal very different relationships.

Significance to Bio-Medical Research and the Program of the Institute: This program should be evaluated in relation to project HD-AG 16 (8)(c) "Modification of Cardiac Arrhythmias through Voluntary Control."

The studies in this program are designed to get at the basic physiological and psychological mechanisms underlying the voluntary control of cardiac function. All animal studies on the voluntary control of autonomic response have been done in lower mammal, and they have usually been acute studies. The value of the program described in this report derives from the facts that (a) it is being done in primates and (b) the preparation is chronic.

We anticipate that the results of this project will ultimately reveal aspects of the function of nervous and cardiovascular systems which will be of great value in understanding the mechanisms underlying autonomic voluntary control. We further anticipate that these studies will be helpful in the design of new procedures with patients. Finally, when we begin to study older animals, we believe we will accumulate a great deal of fundamental information on the role of aging on the nature of nervous and cardiovascular function.

Proposed Course of Project: Testing will be completed for the two animals now in the study, and the fourth and final animal will be tested.

On July 1 an additional professional (Clinical Associate) will be added to this program, and he will conduct studies designed to analyze the roles of specific areas of the brain in mediating the responses we are measuring.

In addition, studies will be initiated to determine the effects of chronic training schedules on cardiac function. Also, if time allows, we will add measurements of cardiac output to those responses which are already recording.

Honors and Awards: Dr. Engel was appointed Associate Professor of Behavioral Biology at The Johns Hopkins University, School of Medicine.

Dr. Engel is President of the Society for Psychophysiological Research.

Publications: Engel, B.T. and Gottlieb, S. H.: Differential operant conditioning of heart rate in the restrained monkey. Journal of Comparative Physiological Psychology. 73: 217-225, 1970.

Table 1: Ratio of successful to total number of training sessions. During slow and fast sessions success is measured relative to baseline HR. During "Alternate" sessions success is measured in terms of the relationship of HR during the fast portion of the training session to HR during the slow portion.

			Differential training		
Subject	Slow training	Fast training	Alternate	Slow	Fast
FP	17/19	7/18	21/23	18/23	5/23
WF	3/17	10/13	22/24	5/24	24/24
MW	15/20	10/20	22/25	17/25	13/25
WL	12/12	14/16	10/10	10/10	9/10
EW	13/27	4/11	15/15	3/15	15/15

Serial No. HD-AG14 (8) (c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Relationships between cardio-respiratory activity, behavior and aging.

Previous Serial Number: Same.

Principal Investigator: Bernard T. Engel (20% of time).

Other Investigators: Reginald E. Quilter

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	.90
Professional:	.60
Others:	.30

Project Description:

Objectives: The purpose of this project is to study physiological and behavioral integration and the effects of aging on these processes.

Methods Employed: Several reports have appeared in the scientific literature during the past few years which indicated that reaction time varies systematically as a function of the phase of the cardiac cycle in which the stimulus to respond appears. Other studies have reported similar phenomena in relation to the breathing cycle. During the previous year we attempted to replicate these findings adding a number of controls which had been excluded in the earlier studies. Our findings were completely negative.

Since reaction time is a complex phenomenon involving perceptual, cognitive and motor components this study attempted to simplify matters by studying the relationship between cardiac cycle, breathing cycle and visual perception. Visual perception was chosen because it involves perceptual and cognitive acts but no motor requirements.

Each subject was asked to view a screen on which one of six characters was flashed through a tachistoscope. The subject was shown the characters in advance so that he knew what they were. Futhermore, the characters

were matched pairs: M and W, Z and N, 9 and 6. Each subject was pretrained to familiarize him with the procedure, and his perception threshold was established. During subsequent testing the stimulation parameters were set for an expected level of about 50% accuracy. During the actual testing each subject received 12 presentations of each character. These 72 presentations were equally divided among six conditions: The P-R interval, the QRS phase or the T wave phase of the cardiac cycle during inspiration and during expiration. A total of ten subjects, five men and five women, were tested.

Major Findings: Table 1 lists the major results of the study. Statistical analyses of these data confirm what visual inspection suggests. There was no systematic relationship between correct responses and phase of the cardiac or breathing cycle.

Significance to Bio-Medical Research and Program of the Institute: The primary purpose of this project was to relate behavioral responses to physiological responses in order to understand better the mechanisms by which aging affects behavior. To date we have been unable to confirm the presence of any relationship among the behavioral and physiological responses we set out to study. The basis question of how aging affects behavior remains, however, it seems likely that new techniques will have to be evolved to assay this question.

Proposed Course of Action: A copy of the report summarizing our findings on the relationship among cardiac cycle, breathing cycle and reaction time was sent to Dr. Enoch C. Callaway, III, one of the first scientists to report a relationship among these variables. Dr. Callaway has accepted an invitation to visit our laboratory to collaborate on experiments designed to reconcile the discrepencies between his results and ours. Pending Dr. Callaway's arrival, no further work is planned on this project.

Honors and Awards: None

Publications: Engel, B.T. Specificity of responses: Response specificity. (Chapter 14 in) Greenfield, N. and Sternbach, R.

Handbook of Psychophysiology. New York. Holt, Rinehart and Winston, (In Press).

Table 1. Number of correct responses as a function of breathing cycle phase and cardiac cycle phase.

	Inspiration			Expiration		
	P-R	QRS	T	P-R	QRS	T
Men	32	35	38	31	39	37
Women	39	42	42	32	33	31

Serial No. HD-AG16 (8) (c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Modification of cardiac arrhythmias through voluntary control.

Previous Serial Number: Same.

Principal Investigator: Eugene R. Bleecker (90% of time).

Other Investigators: Bernard T. Engel

Cooperating Units: Baltimore City Hospitals.
The Johns Hopkins Hospital.

Man Years:

Total:	1.95
Professional:	1.50
Others:	.45

Project Description:

Objectives: 1. To investigate whether patients with atrial fibrillation can be trained to speed and slow ventricular rate (HR). 2. To determine whether clinically significant changes in HR variability can be achieved in patients with this arrhythmia. 3. To identify some of the autonomic nervous system mechanisms which mediate the operant control of HR in patients with atrial fibrillation.

Methods Employed: Six volunteer patients with atrial fibrillation were recruited from The Baltimore City Hospitals and The Johns Hopkins Hospital cardiac clinics. Each patient was under 65 years old, had a history of rheumatic valvular heart disease and was free of serious neurological disorders. During the three week period of study each patient was hospitalized on the Gerontology Research Center research ward of Baltimore City Hospitals.

Each patient was trained in the laboratory to speed and to slow his HR. In addition, two of the patients were trained to decrease the beat-to-beat variability of their ventricular rates.

During a typical experimental session the patient lay in a hospital bed in a semi-reclined position. Arrayed in front of him was a vertical

display of three lights. The top light was red, the middle light was yellow and the bottom light was green. Each session was divided into two phases. Phase 1 was a 512 second baseline period during which automatic equipment monitored and recorded the frequency of his ventricular contractions. Phase 2 was a 1024 second training period during which the patient tried actively to control his HR.

If the patient was being trained to speed his heart, the green light would be turned on and would burn steadily throughout the training period. (No lights were on during the baseline period). When the patient speeded his HR above his baseline rate, the yellow light would go on and remain on as long as HR was greater than baseline rate. As soon as his HR fell below baseline rate, the yellow light would go off. Each patient was instructed to keep the yellow light on as much as possible. He knew that speeding his heart was the correct way to do this.

During the slowing training the procedure was identical to that outlined above for speeding except that the red light rather than the green light burned steadily, and that relatively slow HR now controlled the yellow light.

After the patient had been trained to slow his HR and to speed his HR, he was further tested under differential training conditions. During the training phases of these sessions the patient was required to alternately speed and slow his HR over four consecutive 256 second intervals. The onset of each interval was signalled by the red and green cue lights, and the patient knew that these lights meant that he should try to slow and speed respectively.

During training to control HR variability, the yellow light would be on as long as the patient maintained his HR within predefined limits. As soon as the patient speeded his heart above the upper limit, the red light would go on and the yellow light would go off. Similarily, if the patient slowed below the lower limit, the yellow light would go off and the green light would go on.

Major Findings: Five patients have been studied and the sixth is now being tested. Table 1 summarizes the performances of these five patients.

MW and WL were able to consistently slow and speed HR. FP was able to slow HR reliably, however, she was unable to speed HR consistently. WF and EW were both able to speed HR consistently but were less consistent in their abilities to slow. WF was able to increase his HR about 30% above his baseline rate, and EW increased her HR as much as 60% above her baseline rate. Frequently EW not only increased her HR but also changed her cardiac rhythm such that she would have frequent burst of premature ventricular contractions, often in a bigeminal rhythm. These ectopic beats were almost exclusively limited to periods when she was voluntarily speeding her beat.

Drug studies designed to elucidate the mechanisms of voluntary HR control were performed in FP, WF, MW and EW. Atropine (.05 mg/kg IV) markedly decreased FP, WF and MW's abilities to modify their HRs, however, EW was able to modify her HR with an equivalent dosage. Neither isoproterenol (0.5-2.0 ug/Min. IV) nor propanalol (.075 mg./kg IV) affected the abilities of these patients to modify their HRs. Futhermore, propanalol, which is often used to control ectopic beats, did not prevent EW from producing ectopic beats only during voluntary HR speeding.

WF and WL were given extensive training to try to teach them to decrease the variabilities of their ventricular rates. Although both patients were usually able to reduce their HR variabilities with respect to baseline levels, neither showed any evidence of day to day reduction in variability. Thus, there is no evidence that voluntarily produced, clinically significant changes in HR variability are possible among these patients.

The results of this study indicate that :1) patients with atrial fibrillation can control their HR, and therefore, that ventricular rate is probably neurally regulated in this arrhythmia and not random as some investigators believe; 2) the neural control of HR among these patients is probably mediated at the level of the atrio-ventricular node; 3) the neural control of HR among these patients is probably primarily mediated through the vagus nerve.

Significance to Bio-Medical Research and the Program of the Institute: This project is designed to increase our understanding of the neural mechanisms of cardiac control, especially as these relate to our knowledge of the extent to which it is possible to effect such control voluntarily. These findings add further credence to the conclusions that responses mediated by the autonomic nervous system can be brought under voluntary control; and that it is possible that once such control is attained, it may have clinically useful applications.

Proposed Course of Project: 1) Selected patients with cardiac arrhythmias associated with the atrio-ventricular node will be studied to learn more about the neural control of this region of the heart. 2) Patients with angina pectoris will be studied to determine whether they can significantly control their cardiac function, and thereby, the severity of their symptoms.

Honors and Awards: Dr. Engel lectured (by invitation) to the staff of the Department of Psychiatry, University of Maryland (October, 1970).

Dr. Engel lectured (by invitation) to the staff of the Eastern Pennsylvania Psychiatric Institute (February, 1971).

Dr. Engel lectured (by invitation) to the staff of the Department of Psychology, Elizabethtown (Pennsylvania) (April, 1971).

Publications: Weiss, T. and Engel, B.T.: Operant conditioning of heart rate in patients with premature ventricular contractions. Psychosomatic Medicine (In Press).

NICHD Annual Report
July 1, 1970 through June 30, 1971

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Number: NIH 70-2296
Contract Title: Twenty-five year follow-up analysis among former Army Air Force cadets.
Contractor: University of California (Los Angeles)
Money Allocated: $25,578 (FY 1970)

Objectives: In 1944 Dr. Wenger carried out a series of psychological and physiological tests on a group of 2111 Army Air Force cadets. In 1964 he sent health questionnaires to 1032 of these men and received 725 responses. On the basis of an analysis of these results it appeared that the 1944 tests might predict the occurence of some diseases among these men.

The purpose of this contract is to enable Wenger to carry out a survey of as many of the 2111 men as he can locate to determine the value of the 1944 test battery in predicting the subsequent development of certain psychological and physiological disorders. The reason this contract was initiated through this laboratory is that if the Wenger test battery does have predictive validity we would propose to include it in the longitudinal study program of the Gerontology Research Center.

Major Findings:

1. Administration

The contract was approved on April 29, 1970 by the contract review committee with the contingency that: "An advisory committee acceptable to the Project Officer (Dr. Engel) be established to recommend revision and improvement of the questionnaire to include such items as; data on smoking, the Cornell Medical Index occupational mobility, and identification of respondent's personal physician or hospital for access to medical history (in the event a follow-up is required for data confirmation)."

On June 9, 1970 this contract was negotiated on a cost-reimbursement basis in the amount of $25,578 for a period of one year effective June 30, 1970.

In order to improve the questionnaire and to facilitate subsequent data analyses Wenger added Dr. Leo G. Reeder, Director of the UCLA Survey Research Center, as a contract representative. Dr. Reeder assumed the primary responsibility for revising the original Wenger questionnaire. On August 21, 1970 the revised questionnaire was received by the Project Officer,and on August 27, 1970 the copies of the revision and of the original questionnaire were sent to three outside reviewers,each of whom had been selected because he was trained in internal medicine and because he had done extensive research on the role of behavioral factors in physiological disorders. On October 12,

1970 the Project Officer sent a summary of the reviews to Wenger who returned his revision of the questionnaire on October 26, 1970. On October 30, 1970 the Project Officer notified Wenger of his approval of this version and on November 6, 1970 the Contract Officer was authorized to submit the contract for final Bureau of the Budget approval which he did on November 12, 1970. Wenger did not receive notification of Bureau of the Budget approval until February 10, 1971.

2. Summary of results to date:

A. Location of subjects	
1. Total samples	2111
2. Men with V.A. Claim files	1861
3. Others traced through Military Personnel Center of the National Archives and Research Service	221
4. Subjects for whom no addresses are yet available	19
B. Mailing data (3/19-3/31)	
1. Subjects known to be dead	135
2. Questionnaires completed	301
3. Mailings returned as "undeliverable"	537
4. Mailings for which no responses yet exist	588
5. Subjects to whom questionnaires have not yet been sent	550

At this stage of the project four operations are going on simultaneously.

1. Efforts are being made to find addresses for the remaining 19 subjects.
2. Efforts are being made to find forwarding addresses for the 537 questionnaires which were returned as undeliverable.
3. V.A. claim files and other data are being reviewed on the 550 subjects who have not been mailed questionnaires to determine current mailing addresses and other personal data.
4. Questionnaires from the 301 respondents are being reviewed, and the responses are being key-punched for subsequent computer analysis.

Significance to Bio-Medical Research and Program of the Institute:

The importance of this survey is intuitively obvious. The sample of about 2000 men on whom pre-test data exist is unique. If it can be shown that these pretests can predict the emergence of subsequent diseases, then these tests will be important additions to the longitudinal study program of the Gerontology Research Center. It seems highly likely that if a single test battery can predict disease after a 25 year lacuna, repeated testing such as would be possible within the longitudinal study program would increase the predictive value of the tests even more. If it is possible to identify persons who are likely to develop diseases at later stages of life, it should also be possible to develop and

institute preventive procedures early in life. And increasing the productive, healthy portion of the human life span is the primary goal of the Gerontology Research Center.

Proposed Course: The primary task within this project at this phase is to locate as many of the subjects as possible. Once that has been done and the questionnaire responses have been key-punched, the next step will be the statistical analyses of the data. Depending upon the outcome of this step, it may then be necessary to contact personal physicians among the respondents to confirm the presence and natural history of specific diseases.

Serial No. HD-AG7 (c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Section on Human Learning and Problem Solving
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Verbal Learning and Age.

Previous Serial Number: Same.

Principal Investigator: David Arenberg (50% of time).

Other Investigators: Walter W. Surwillo, University of Louisville.

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	1.90
Professional:	.50
Others:	1.40

Project Description:

Objectives: General objectives are to discover important conditions which affect age differences in acquisition and retention of verbal material in man for two purposes: (1) an understanding of the factors which enable us to modify age differences experimentally should provide valuable information about aging processes and about psychological processes of learning; and (2) an understanding of the conditions which are particularly beneficial or deleterious to learning by the old may have implications for training of old workers.

Early studies in the project explored the effects of variation of time periods in acquisition of verbal material. Recent and current studies were designed to evaluate the effects of other variables, particularly the beneficial effects of augmenting visual presentation of various material to be learned.

Methods Employed: Participants in the experiments described below were recruited from local non-profit employment services, high schools, and colleges and were paid to participate. They did not have college degrees and many had not graduated from high school. They were men between 17 and 23 or between 60 and 70 years of age. Most of the men from the employment services were (or had been) blue collar workers.

Virtually all of the men from sources other than the colleges were of average or low-average intelligence.

Although, many different procedures have been used in the studies described below, two general methods can be specified: (1) rote verbal learning and (2) retention of material presented only once. In the rote studies, material is presented many times and the major focus is on improvement in learning or acquisition. In all the other studies, material is presented once and the focus is on retention.

Experiment XIV was designed to investigate the effects of auditory cues (in the form of verbal descriptions) on retention of geometric figures. This is not a verbal retention task, but it was of interest to determine whether auditory augmentation known to improve retention of verbal material would also improve retention of perceptual material. The two presentation conditions were: (1) visual- the material was exposed for 12 seconds and 15 seconds later the subject attempted to reproduce the figures, and (2) auditory-visual- same as visual except that the salient features of the figures were described during the 12-second exposure. All subjects reproduced two sets of seven designs (each design consisted of three figures). One group performed on both sets under the visual condition; the other group performed under the visual condition, then the auditory. The effects of verbal description during visual display were evaluated by comparing change in performance for the two old groups and the two young groups.

Experiment XVIII is a rote paired-associate learning study with the three input conditions used in short-term and free-recall studies in this series (Experiments XII, XIII, and XV). Acquisition trials are alternated with self-paced test trials. During the acquisition trials, subjects (1) look at the items, (2) look at the items and listen to the experimenter read them aloud, or (3) read the items aloud. Performance during identical test trials is compared for the three conditions. Unlike the other studies using these three input conditions, each subject is randomly assigned to one of the conditions and he learns one set of letter-word pairs.

Experiment XX is a further exploration of input and output interference. As in the earlier study (Experiment XIX), half messages consist of four digits or four letters and the presentation order of half messages is systematically varied. Similarly, response halves can be specified and response order varied systematically. Four possible combinations of input and output interference result for digits and for letters. For example, if an item is presented letters first, (digits second) and letters are specified to be recalled first, the letters are said to be subject to input interference (from subsequent presentation of the digits) but not to output interference; the digits are subject to output interference (from prior recall of letters) but not to input interference. The response can be immediate or delayed for four seconds. In Experiment XX, the delay interval can be unfilled (as in Experiment XIX) or filled by a color matching task designed to be minimally interfering with recall, but sufficiently demanding to prevent rehersal

of the digits and letters. In addition, the apparatus provides an accurate time measure for each element in a response. Responses are key presses rather than written (as in Experiment XIX).

Experiment XXI is another free recall study designed to specify the source of interference discovered in Experiment XV. In that earlier study, it was found that recall of words presented at the beginning of a list is impaired by hearing or saying all the words in the list. By systematically varying within a list which words are spoken and which are only seen, it should be possible in Experiment XXI to determine whether saying interferes with recall of the spoken word or with recall of previous words.

Experiment XXII was a collaborative study with Dr. Walter W. Surwillow of the University of Louisville. In this experiment, lists consisted for four, six, or eight digits presented two at a time. The two simultaneous digits were presented one to each ear using stereophonic ear phones. This procedure is called dichotic presentation. Some time ago, it was reported that correct responses of such items are recalled in ear order, i.e., all the digits presented to one ear are recalled before any of the digits to the other ear. Many dichotic experiments have since been reported in the literature, but the incidence of the effect under various conditions has not been compared with the incidence expected by chance. The primary purpose of the current experiment was to make this comparison in several samples using three list lengths.

Experiment XXIII is another study of the effects of auditory augmentation on recall of visually presented geometric figures. In a similar study (Experiment XIV), it had been found that hearing descriptions of designs as they were seen improved retention, particularly for the old. In Experiment XXIII, the procedure is similar, but the designs were made to minimize the ceiling effect (near perfect performance) for many of the young subjects, and to be equivalent to permit comparison of practice effects for both age groups under both conditions.

Major Findings: The final results of Experiment XIV were the same as the preliminary results reported last year. Retention of visually presented geometric figures was improved when the salient features of a design were described by the experimenter (on tape) while a subject inspected the design. Auditory augmentation was particularly effective for the old; but many of the young committed so few errors, there was little room for improvement. Experiment XXIII was designed to reduce the ceiling effect and to provide comparable design which permit assessment of improvement with practice.

In Experiment XVIII, the effects of saying or listening to visually presented items in a rote (repeated) task are investigated. Earlier studies in this series had shown that auditory augmentation (saying or listening) improved recall of material just presented but was detrimental to recall of words presented at the beginning of a list. Pre-

liminary data for the three young groups in Experiment XVIII suggest that listening (and looking) is superior to saying and to just looking. Thus far, it seems that for young men, in a rote learning task in which material is presented many times, hearing the words enhances learning, but the additional activity involved in saying the words offset the beneficial effects of hearing them.

Experiments XX, XXI, and XXIII are at about the same stage of development. Experiment XX is the short-term-memory study in which the delay interval is occupied by a minimally interfering task and the timing of individual response elements can be measured. Experiment XXI is the free-recall study in which the source of the interference from saying words early in a list is investigated is the new study of retention designs with and without auditory cues. In all three studies, apparatus has been constructed and modified, stimulus material has been prepared, and data collection is just beginning.

Experiment XXII was the dichotic retention study in which the incidence of ear-order recall was compared with chance incidence. Dichotic items are presented in simultaneous pairs with different messages to each ear. At all three list lengths, the group proportion of ear-order responses exceeded the proportion expected by chance, but of greater interest were the distributions of individual proportions. Most subjects fell into one of two classes: (1) those who never responded in ear order, and (2) those who responded in ear order far beyond chance levels. In addition, for lists in which the message to one ear was recalled correctly but an error occurred in the other, a high proportion of correct messages were recalled first.

Significance to Bio-Medical Research and the Program of the Institute: Learning is more central to experimental psychology than any other behavior of higher organisms and some of the most striking and consistently reported behavioral age differences in the Gerontological literature have been found in verbal learning performance. The experiments in this project are designed to identify basic mechanisms of learning and retention and to measure changes in these functions that occur with age.

Aging, viewed as a natural experiment in which many changes occur, permits one to identify a variety of processes which may be important underlying mechanisms of learning. In addition, knowledge about experimental variables which affect age differences will be valuable in developing techniques for optimizing learning of the older person.

Proposed Course of Project: Data collection will continue for Experiments XVIII, XX, XXI, and XXIII, and for the longitudinal studies of learning and retention. A manuscript describing Experiment XXII has been written and will be submitted for publication. In addition, attempts will be made to prepare dichotic tapes in order to pursue the research questions which came out of Experiment XXII. We hope to explore such questions as: What characteristics discriminate ear-order

responders from sequential responders? and What experimental conditions affect incidence of ear-order and sequential responding?

Honors and Awards: Dr. Arenberg was invited to the faculty of of the University of Southern California, Summer Institute for Advanced Study in Gerontology, July, 1970.

Dr. Arenberg was invited to participate in a symposium on "Memory and Thinking" at the meeting of the American Psychological Association, September, 1970.

Publications: None.

Table 1. Distribution of 41 Ss by probability of each individual's incidence of ear-order recall occurring by chance for lists presented dichotically.

Probability decile	Number of Ss
.1 or lower	18
.1 to .2	5
.2 to .3	2
.3 to .4	1
.4 to .5	0
.5 to .6	0
.6 to .7	1
.7 to .8	0
.8 to .9	3
.9 to 1.0	11

Note: These tabled values are based upon the probability (combined over list lengths) that the number of lists recalled in order (or more) would occur by chance.

Serial No. HD-AG6 (c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Section on Human Learning and Problem Solving
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Problem Solving and Age.

Previous Serial Number: Same.

Principal Investigator: David Arenberg (50% of time).

Other Investigators: Phillip Thorne

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	1.75
Professional:	0.50
Others:	1.25

Project Description:

Objectives: The general goals are to explore and identify reasoning processes in man, to determine in what ways these processes change with age, and to develop techniques for reducing age deficits in reasoning performance. In this project, reasoning is studied by using problem-solving procedures in which on-going solution behavior can be observed and quantified. Experiments are designed to answer such questions as: (1) Is effectiveness in acquiring relevant information affected by aging? (2) Is effectiveness in synthesizing available information affected by aging? (3) What kinds of solution strategies are used and in what ways are they related to age? (4) How does imposing a memory load affect solution strategies for young and old adults?

Methods Employed: Modifications of the Problem Solving and Information Apparatus (PSI) are used in Experiments I and IV. The display board for all problems includes nine lights on the periphery of a circle and a goal light in the center. The peripheral lights can be turned on by switches. A subject's task is to light the goal light in a prescribed way. For each problem a disk is superimposed on the light display panel. On the desk are arrows connecting various lights. An arrow indicates that one of three logical relations (previously explained to the subject)

exists between the lights that arrow connects, but the specific relation is not identified. The solution of each problem requires lighting the goal light by a sequence of inputs[a] in which only the switches for a specified set of three (of the nine) peripheral lights can be used. Time between inputs is determined by the subject. In addition to correctness of the solution, performance is evaluated by determining whether an input (at the time it is made): (1) provides new information, (2) provides inferrable information, or (3) is overtly redundant. For these two studies using PSI problems, subjects are participants in the GRC longitudinal program.

[a]The term "input" refers to the situation in which one or more lights are on and a subject indicates he wants the results of those lights. Those lights are then extinguished and, according to the logical relations of the arrows pointing away from those lights, their resultant lights are turned on.

Another general type of problem used in this project is concept identification. In a concept identification problem, the task is to determine the concept, i.e., the attributes which define a set. Information is obtained from examples which are labeled positive (an example of the concept) or negative (not an example of the concept). In an earlier study of concept identification (Experiment III in which examples were selected by the experimenter and concepts were simple, i.e., one attribute defined the concept), old solved fewer problems than young. Other studies in the literature had not found such age differences, but those studies matched age groups on non-verbal intelligence. Experiment IIIa was designed to explore the effects of such matching.

Experiment V is a concept identification study with longitudinal participants. In this study, subjects (rather than the experimenter) select the examples. This procedure provides a measure of information gain for each selection and can also indicate the kinds of strategy used to solve problems. Ordinarily, when subjects select examples, logically equivalent selections do not result in equivalent information gains, and fortuitous selections can drastically alter the difficulty of a problem. A technique was developed which provides equivalent information gains for logically equivalent selections and avoids fortuitous gains in information. This technique also has two other important characteristics: (1) different types of problems which are logically equivalent can be constructed and compared, i.e., conjunctive concepts (positive examples require joint presence of two attributes) can be matched with two-attribute disjunctive concepts (positive examples require presence of at least one attribute); and (2) information gain can be manipulated because the experimenter can minimize or maximize the information gain for each selection. Experiment V was designed to explore age differences and age changes among different types of problems and the effects of varying amount of initial information gain.

Experiment VI is a concept identification study designed to explore an unexpected finding emerging from Experiment V. Preliminary results of Experiment V had shown that when initial information was high, dis-

junctive concepts were identified more effectively than conjunctive; in the literature, conjunctive concepts have consistently been identified more readily than disjunctive. Variations in procedure were introduced to determine whether procedures specific to Experiment V contributed to that unexpected finding. Several procedures were changed, but the major changes involve the presentation of the feedback message (whether an example is positive or negative). In Experiment V, problems are described as poisoned-food problems and the task of the subject is to identify two poisoned foods. Subjects' selections are referred to as "meals" consisting of four "foods" and the feedback messages are "died" (positive) and "lived" (negative). Communication between subject and experimenter is verbal. Subjects use paper and pencil and the experimenter uses a special purpose digital computer to determine appropriate designations (positive or negative) of subjects' selections. In Experiment VI, switch problems logically identical to the conjunctive and disjunctive poisoned-food problems of Experiment V are used. Each selection consists of four switch positions. The feedback messages in conjunctive problems are "Both in" (positive) and "Not both in" (negative); in disjunctive problems the messages are "At least one in" (positive) and "Neither in" (negative). Subjects' selections are presented directly to another special purpose digital computer which immediately provides the appropriate feedback. In both Experiments V and VI, subjects are instructed to maintain a complete written record of their selections and the feedback for each in order to minimize the memory component of the task.

Major Findings: Preliminary analyses of longitudinal data in Experiment I, based on 93 subjects each of whom was retested at least six years after the initial performance, show: A. For the first problem, (moderately complex): (1) There were few failures to solve in any age group either time. (2) There was little change in effectiveness for the group below 45 initially (N=28) or for the group between 45 and 60 initially (N=42); but for the over-60 group (N=23) there was a small decrement, mostly due to seeking information already inferable from previous inquiries. B. For the second problem (somewhat more complex than the first): (1) There were few failures to solve among the men below 60 initially; but there was a substantial number of failures to solve among the men over 60 (5 of 16 failed the first time, 7 of 16 on retest), despite the select subsample of men who reached this problem (the men who reached this problem were predominantly good performers on the first problem). (2) There was slight improvement in effectiveness for the men below 45 initially (N=27), little change for the men 45 to 60 (N=40), but substantial decrement among the men over 60 (N=16) characterized partly by an increase in seeking already inferable information (similar to the first problem) but mostly by an increase in overt repetitions of inquiries.

Experiment IIIa is a study of concept identification among men of average and low average intelligence to determine whether matching old and young groups on non-verbal intelligence affects performance differences between them. Preliminary data show that an old group (60-70) matched with high school students (17-19) solved as many prob-

lems as the young. An unmatched group of 60-70 year old men solved fewer problems than the young, results similar to an earlier experiment in this project. Prior to that earlier study, the few age studies of concept identification in the literature had matched age groups and had reported no age differences in number of problems solved. It is beginning to appear from the results of the current experiment that matching groups serves to select the better problem solvers among the old.

Data collection continues for Experiment V, a longitudinal study of concept identification, but now the more complex problems are administered using the Gerontology Research Center general purpose computer on-line. The computer indicates the appropriate feedback message for each inquiry selected by a subject. The feedback is based upon a set of rules selected by the experimenter and the subject's previous selections in the problem. In addition, the computer stores the sequence of selections during the solution of the problem, and later (off-line) assesses each selection (based upon the information available to the subject at that point in the problem) and the total solution on several measures.

The on-line feature of the general purpose computer also permits a broader range of complex concept-identification problems to be used in Experiment VI and subsequent experiments.

Significance to Bio-Medical Research and the Program of the Institute: Reasoning is among the most prized behaviors of man and among the most elusive for experimental study. In this project, methods have been and will be developed for obtaining quantifiable measures of step-by-step performance on reasoning problems. Some of these methods also provide patterns of responses which represent strategies in solving such problems.

Measures are obtained in current experiments to study changes in reasoning processes with age. These studies, in addition to identifying basic reasoning processes, should indicate how pervasive reasoning deficits are with age, whether education and cognitive activity mitigate such deficits, and what techniques could be used to minimize decline in reasoning.

Proposed Course of Project: Data collection will continue for Experiments IIIa and VI and all the longitudinal studies. A new study of concept identification using patterns as stimuli has been designed and will be implemented on-line with the computer when a visual output device becomes available.

Honors and Awards: Dr. Arenberg was an invited faculty member of the Institute for Research and Teaching in Gerontology sponsored by the Gerontological Society, August, 1970.

Dr. Arenberg was appointed Associate Editor of the Journal of Gerontology.

Honors and Awards:

Dr. Arenberg was invited to contribute a review paper for the American Psychological Association Task Force on Aging.

Publications: None.

Serial No. HD-AG (8) (c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Section on Human Learning and Problem Solving
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Perceptual Retention and Age

Previous Serial Number: Same

Principal Investigator: Dr. Arenberg (0% of time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

No research was conducted this reporting year, but the project will resume in 1971 to 1972.

Serial No. HD-LB 1
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Behavioral Genetics and Aging.

Previous Serial Number: None.

Principal Investigator: Charles L. Goodrick (85% of time)

Other Investigators: None.

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	1.85
Professional:	1.70
Others:	.15

Project Description:

Objectives: The principal objectives of this project are to analyze inbred strains of mice to determine for behavioral traits and longevity: (1) Heritability (degree of genetic determination vs. degree of environmental determination; (2) Mode of inheritance (e.g. over-dominant, dominant, intermediate, or recessive); and (3) Number of segregating units (gene blocks) controlling a particular trait (e.g. longevity). Another objective is to determine the relative behavioral differences among strains as aging progresses.

Other objectives include: 1) The development of an animal model with the chemical and behavioral characteristics of human phenylketonuria; 2) Analyses of the influence of diet (e.g. casein) on behavioral traits, growth, and longevity; and 3) Determination of single-gene influences upon behavioral traits, growth, and longevity.

Methods Employed: Inbred mice (C57BL/6J and A/J) of a high degree of homozygosity are maintained under uniform environmental conditions. The animals are tested behaviorally during one period of their life span, e.g., when mature, mature-old, or senile. Senility is determined as the 50% mortality point for groups maintained throughout their life span. Statistically reliable techniques have been developed to deter-

mine behaviors relevant to natural selection such as exploration, general activity level, emotionality, simple or complex problem solving ability, and taste preference. The use of segregating groups allows an estimate of the mode of inheritance, e.g., dominant or intermediate, and the number of gene blocks or segregating units controlling behavioral traits or life span.

Rats tested to find an animal model of phenylketonuria were injected in infancy with either saline, phenylalanine hydroxylase or p-chlorophenylalanine and behavioral tests were conducted at maturity.

The diets used for studies in which protein intake was varied among groups of inbred and hybrid mice utilized isocaloric synthetic casein diets obtained from General Biochemicals, Chagrin Falls, Ohio. Deprived animals received 4% casein in their diets whereas the control group received 26% casein in its diet.

Numerous kinds of mutant mice are maintained on the C57BL/6J background at the Jackson Memorial Laboratories, Bar Harbor, Maine. So far, our work has concentrated on the albino mutant at the c-locus, Linkage Group 5.

Major Findings: A. Cross sectional behavioral studies of aging populations of inbred, hybrid, and F_2 mice.

An experiment has been completed to determine the effects of age and genetic constitution upon behavior and longevity for A/J and C57BL/6J inbred mice. A/J mice are albino (white coat), short lived, behaviorally inactive mice, while C57BL/6J mice are melanic (black coat), long lived behaviorally active mice. Both groups die of nonspecific causes. Groups of A/J, C57BL/6J, F_1, and F_2 mice have been tested on a number of behavioral measures with an N at least 16 per group at 5, 14, and 23 months of age, and at the time of advanced old age for the four groups: A/J (23 mo.), C57BL/6J (26 mo.), F_1 and F_2 (29 mo.) at the approximate time of senility or 50% mortality. Behavioral data were collected using standard tests developed in our laboratory to determine: (1) sensory discrimination, (2) preference for ethyl alcohol, (3) performance and retention of a learned operant response, (4) exploration behavior, and (5) emotionality. These data are in the process of analysis.

Longevity data are now available based on populations of 100 each of A/J, C57BL/6J, F_1, and F_2, mice (Table 1). Groups of 50 male and 50 female mice were combined because sex differences in longevity were not obtained.

The data indicate a dominant mode of inheritance for longevity with over-dominance in the F_1 generation. Heritability was found to be .787, while just one gene unit was estimated as responsible for the mean parental differences in longevity. Behavioral analyses are now in progress.

B. An animal model for the study of phenylketonuria.

A series of experiments have been completed and data analyzed for populations of rats injected during infancy with saline (S), phenylalanine hydroxylase (Ph), and p-chlorophenylalanine hydroxylase (Pcp), to determine whether an animal model of phenylketonuria (PKU) could be simulated in rats. The animals were injected in infancy by Dr. G. Guroff and maintained by him until maturity. PKU in human populations is a hereditary disease, transmitted as a simple autosomal recessive factor. The chemical conditions for the experimental duplication of PKU in rats are: (1) the presence of phenylpyruvic acid in the urine, (2) reduced phenylalanine hydroxylase activity, (3) serum phenylalanine level above about 20 mg. %, and (4) serum tyrosine levels low or equivalent to control animals. Dr. Guroff is working to create the conditions for the chemical duplication of PKU, while we are interested in analyzing the behavioral consequences of his procedures.

There are few guidelines in the selection of behavioral tests in this project. Apart from the obvious intellectual deficit that one sees in human PKU, there are no useful data on such other psychological traits as temperament and sensory discrimination. Accordingly, we are using the tests which have been useful in identifying the behavioral deficits in animal senility to evaluate the behavioral deficits in these animals with hyperphenylalanemia. These tests are: 1)complex problem solving as measured by the Stone multiple-unit T maze; 2) exploration and emotionality as measured in a standardized open field; 3) taste discrimination for sucrose and ; 4) taste preference for ethyl alcohol. The following behavioral results were obtained. a. Exploration behavior: group differences were not statistically significant, although Pcp groups were more active than S or Ph groups. b. Emotionality: the Pcp group was less emotional than S or Ph groups ($F(2,54)=3.14$, $p < .06$). c. Sensory discrimination: group differences were not statistically significant. d. Alcohol preference: group differences were not statistically significant. e. Complex maze learning: the Pcp groups made more errors during the final learning trials than controls, and in general made more errors than control rats ($F(1,38)=4.45$). The results show that Pcp animals differ from senile old animals in most of the behavioral traits measured. Old animals explore less than young ones, they are more emotional, they have poor sensory discrimination and show less preference for ethyl alcohol. The only behavioral deficit which Pcp and senile animals share is in problem solving ability. Thus, it would appear that Pcp pretreatment rather specifically diminishes intellectual behavior.

C. The effects of diet on growth and behavior.

This project was designed to assess the effects of low or normal protein intakes during various stages in development upon rate of growth and behavior at maturity for genetically homogeneous groups of mice. Groups of A/J, C57BL/6J, and F_1 hybrid mice (N=240, 80/group) were placed on low (L, 4% casein) or normal (N, 26% casein) protein isocaloric diets from 4 to 12 weeks of age, then were split into sub-

groups which were fed either low or normal protein diets from 12 weeks of age. For each group of mice, there were four subgroups (n=20 in each subgroup); LL (low protein diet weeks 4-26 +), LN (low protein diet weeks 4-12, normal protein diet weeks 12-26 +), NL (normal protein weeks 4-12, low protein diet weeks 12-26 +), and NN (normal protein diet weeks 4-26 +). The mice were weighed weekly to determine growth rate, and behavioral tests were conducted after the mice reached the age of 26 weeks to determine emotionality, exploration, and wheel activity. An operant learning test obtained the reaction of the mice to novel stimulation, i.e., light contingent bar pressing, while an extinction measure provided an index of learning.

Results for growth (weight gain) were consistent for all nutritional groups. A/J mice were lowest, with C57BL/6J mice intermediate, and F_1 groups highest in final weight. C57BL/6J and A/J mice reacted differently to the low and normal protein diets when started at the age of four weeks. Normal protein diets resulted in much faster growth of C57BL/6J groups than A/J groups. Low protein diets resulted in initially faster growth for the A/J groups than the C57BL/6J groups. These results demonstrate the importance of genetic constitution in the study of growth responsivity to diets deficient in protein.

Behavioral tests results were (a) mice fed normal diets early in development were more emotional than mice fed low protein diets early in development (F (1,228) = 29.07, $p < .01$), (b) the effect of protein level upon exploration behavior was a function of mouse strain; low protein diets resulted in lower activity scores of a high activity mouse strain, compared with controls, but higher scores for a low activity mouse strain, compared with controls (F (2,227) = 26.39, $p < .01$), and (c) wheel activity was higher for mice fed low protein diets early in development than for mice fed normal protein diets early in development (F (1,227) = 15.07, $p < .01$). Low protein diets compared with normal protein diets fed to mouse groups early in development had no significant effects upon responsiveness to a light onset stimulus during training or extinction tests.

D. Studies of mutant mice.

The effects of a mutation, albinism, at the A-locus of Linkage Group 5, has been studied to determine behavioral differences between mutant albino mice and nonmutant control C57BL/6J mice. Albino mice drank more water, ate more feed, and produced much more food waste than expected on the basis of food intake differences compared with pigmented controls. In addition albino mice built poorer nests and were more aggressive than pigmented mice. These differences in temperamental traits were related to a general hyper-excitability of mutant compared with non-mutant mice.

Groups of albino mutant and pigmented mice were also individually tested for 40 trials (4 per day for 10 days) in a stone 14-unit multiple-T maze. Albino mice made significantly more errors than pigmented mice on test days 3 to 10. Measures of weight loss, speed

of running the maze, and open field exploration were consistent with the hypothesis that motivational drive was not lower for albino than pigmented mice. The error differences demonstrated a single-gene locus clearly related to complex problem solving behavior which was not due to slow performance or inactivity commonly associated with albinism.

Significance to Bio-Medical Research and Program for the Institute: (A) The study of the genetics of behavior and longevity allows an assessment of (1) the mode of inheritance for the factor studied (e.g. dominant, intermediate, etc.), (2) the relative importance of hereditary and environmental factors, and (3) the number of genes or gene blocks which control the factor studied. (B) A chemically and behaviorally appropriate animal model for the study of phenylketonuria is important in the study of methods for the amelioration of the behavioral and physiological characteristics of phenylketonuria. (C) Lack of adequate dietary protein is a condition which affects a large proportion of the world population. Our program attempts to determine the effects of diet (such as different proportions of protein in the total diet), during particular stages of the life span upon behavior and longevity for animal populations which differ in genetic constitution. (D) Studies of single-gene mutant animals are of importance because they allow the assessment of the importance of a specific genetic locus for physiological or behavioral factors.

Proposed Course of Project: A. Further inbred strains and F_1 hybrid groups are being studied to determine the generality of mode of inheritance for behavioral factors. Cross-sectional and longitudinal studies of mouse behavior should continue with mouse strains BALB/cJ and DBA/2J, in addition to the A/J and C57BL/6J mouse strains. The longevity of inbred and hybrid groups should also be determined. B. A further study of the rat model for PKU should emphasize the relationship of complex problem solving to the chemical correlates of PKU. C. Experiments with low and normal protein diets should determine: (1) the relationships between growth rate and longevity, (2) the effect of low, normal, or high protein diets upon behavior at maturity after access to these diets during various stages of development, and (3) the effects of a present diet of low, normal, or high protein upon subsequent behavior. D. Mutant mice on the C57BL/6J background should be studied regarding differences in behavior and longevity.

Honors and Awards: None.

Publications: None.

Table 1

	Groups			
	A/J	C57BL/6J	F_1	F_2
Longevity		Age at death (in months)		
Mean	21.9	27.1	28.5	26.6
Median	23	27	28.5	28
Mode	24	28	31	32

Serial No. HD-AG 25
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Operant Performance, Memory and Aging.

Previous Serial Number: Same.

Principal Investigator: C. L. Goodrick (10% of time).

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	.25
Professional:	.20
Others:	.05

Project Description:

Objectives: Research on both human and animal subjects has shown that short-term memory deficits and motor performance deficits are major problems of aged individuals. An interpretation of maze learning differences between young and senescent rats is that these differences are due to short-term memory deficits of the senescent animals. The general objectives of the problem solving portion of the project are: (1) to analyze complex maze learning of young and senescent animals and (2) to determine variables which may act to enhance or retard maze learning ability. The objectives of the other phase of this project are: (1) to study age differences in motor performance during operant responding, and (2) to develop operant techniques which improve the retention of learned responses in old animals.

Methods Employed: The operant conditioning performance and retention studies have used 2-bar test boxes in which hungry animals are trained to press one bar to obtain a food reward, while the alternate bar remains neutral. By increasing the complexity of the task (using two bars rather than one), it is possible to make a finer analysis of performance and the retention process. We are studying performance and retention as a function of reward schedule, and we are particularly interested in partial reinforcement effect. The retention of partially rewarded responses

is vastly greater than for responses continuously rewarded; an analysis of this phenomenon will provide information regarding the general retention process. A complex 14-unit multiple-T maze is also utilized. This maze has been shown to have a high degree of reliability and has been used in many major studies of aging and learning. Additional mazes of 6-units are being developed to study mastery of consecutive problems by young and senescent rats. These mazes will be used to analyze aging effects in short-and long-term memory, and determine aging effects in proactive and retroactive inhibition. The study of these processes has been hampered by the lack of mazes of comparable difficulty.

Major Findings: An experiment is in progress to examine the retention of learned operant responses as a function of reward schedule for young male Wistar rats. This experiment utilizes 2-bar operant test chambers and is based on our earlier research report last year. Our earlier results were that fixed interval schedules of reward resulted in greater response retention than fixed ratio schedules of reward. The present experiment represents a more systematic investigation of reward schedules by studying other schedules requiring more responses per reward for fixed ratio schedules or more time between rewards for fixed interval schedules than in the earlier research. The format consists of a series of daily trails: (1) preliminary training, (2) training, (3) experimental extinction and spontaneous recovery, (4) experimental extinction and spontaneous recovery with secondary reward, (5) a rest interval, (6) spontaneous recovery, and (7) counter-training. Initial groups have been tested after training on a high fixed ratio schedule (FR25) and comparison nontrained control groups have also been tested. Control groups, although essential for a valid comparison of extinction effects, have not been available in previous experimental studies of extinction. Statistical analyses will be completed when all the groups have completed testing.

Significance to Bio-Medical Research and the Program of the Institute: The major points of significance to bio-medical research and the program of the institute are: (a) a determination of the differences and similarities during operant performance and complex problem solving for immature, young, old, and senile lower animals, (b) specification of the learning and retention process for young and senescent lower animals, and (c) the determination of environmental and/or physiological variables which may act to enhance or retard performance, learning, or retention.

Proposed Course of Project: Further studies are in progress to determine the nature of the partial reinforcement effect in relation to (a) time contingent vs. response contingent partial reinforcement and, (b) massed vs. distributed extinction trials. Other studies will examine age differences in operant performance and retention as a function of response effortfulness. Maze studies will concentrate upon the effects of central nervous system stimulants (such as the amphetamines) upon behavioral rigidity within the maze for old rats.

Honors and Awards: None.

Publications: None.

Serial No. HD-AG 27
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Exercise, general activity level and aging.

Previous Serial Number: Same.

Principal Investigator: C. L. Goodrick (15% of time)

Other Investigators: None:

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	.15
Professional:	.10
Others:	.05

Project Description:

Objectives: The general objectives are to determine (1) methods for increasing physical activity of lower animals, (2) behavioral and longevity differences between animals which differ in physical activity level, and (3) to determine the physiological mechanisms underlying group differences in activity.

Methods Employed: Subjects are placed in activity wheels and allowed limited access to food. The result is an increment in physical activity (running) compared with normally fed animals or control animals with limited access to food, but not placed in the activity wheels. Hungry animals may also be rewarded with food for running. Other studies utilize pure strains of mice or different species which differ in activity level due to different genetic constitutions. (See project concerning Behavior Genetics).

Major Findings: 1. Wheel activity measures were obtained to determine if genetically homogeneous mouse groups which differ in longevity also differ in activity level through their lifespan. Mice were tested individually in activity wheels for a period of ten days. The average of the highest four days was used as an estimate of maximal activity. Groups of mice with short (A/J), intermediate (C57BL/6J) and long (F_1 hybrid) life spans were tested to determine activity level at various stages in

the life span. The mouse group with an intermediate life span, C57BL/6J, was found to have the highest mean activity level (Table 1). Although mean activity of short lived A/J mice decreased between 14 and 23 months of age, the activity level of longer lived groups, C57BL/6J and F_1 mice, remained at a consistently high level. At senescence, all groups had a reduced level of activity. (Senescence was defined as 23 months for A/J mice, 26 months for C57BL/6J mice, and 29 months for F_1 hybrid mice).

A major finding in this experiment was the high level of activity maintained by the long lived groups over the portion of their life span from 14 to 23 months. Thus, long lived animals may have a proportionally longer period of vigorous activity than short lived animals, rather than a proportionally longer period of senility.

Significance to Bio-Medical Research and the Program of the Institute: One of the consistent findings of gerontological research is the decline in general activity level of old animals compared with young animals. It is important to determine whether quantity of activity (e.g. general wheel activity) and/or quality of activity (e.g. increased exploration behavior or greater response variability) may be increased for old and senile animals.

Proposed Course of the Project: Mature-young, or mature-old, and senile rats will be trained to increase base activity level for food rewards to determine: (1) maximum increases which may be obtained, (2) the durability of increases (i.e. if increments in activity are maintained after training ceases), and (3) if increased activity level results in transfer to other tests or conditions. Other species will be tested to determine: (1) correlations between general activity level and longevity and, (2) longevity for exercised and nonexercised groups.

Honors and Awards: None.

Publications: Goodrick, C. L.: Free exploration and adaptation with an open field as a function of trials and between-trial-interval for mature-young, mature-old and senescent Wistar rats. Journal of Gerontology, 26: 58-62, 1971.

Table 1- Mean activity level (x 100, maximal activity/24 hrs.) for A/J, C57BL/6J, and F_1 mice as a function of age.

	Age in months				
Groups	5	14	23	26	29
A/J	34.8	28.2	13.1	-	-
C57BL/6J	80.8	57.3	53.0	28.9	-
F_1	78.2	42.3	46.6	-	17.4

Serial No. HD-AG26

1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Solution discrimination, preference and fluid ingestion of rodents as a function of age.

Previous Serial Number: Same

Principal Investigator: Dr. Charles Goodrick (0% of time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

No work was done on this project in the current year.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Gerontology Research Center
Clinical Physiology Branch

Research in the Clinical Physiology Branch continues to be geared to a physiological and biochemical dissection of mechanisms underlying deteriorative or adaptive aging changes in man. Of necessity the individual studies are at different stages of polish; the function of some organ systems seem to defy sophisticated approaches in man while others are subject to intense development and, in some cases, application.

The Longitudinal Study of the Center provides the essential resource for the investigation of aging in man. In order to maintain 600 active participants of the appropriate ages, new recruitment in the past year has been limited to the very youngest adult subjects (who must be replaced as they graduate into middle-age) and the very oldest subjects (where losses are especially high due to mortality and illness).

In the past year advances have been made in data retrieval and computer programming so that very informative cross-sectional and longitudinal analyses of multiple variables are being provided to the responsible investigators. Valuable "stem-and-leaf" arrays of distributions of variables in selected age groupings are part of this routine as well as automatic read-out of selected percentiles in these distributions. Standard statistical computation of means, standard deviations, etc. are provided for these age groups and regressions of experimental variables on age are also routine. In addition true longitudinal analysis of specified variables in each individual subject is now automatically provided.

Classification of subjects with respect to the prevalence and incidence of atherosclerosis in coronary and cerebral arteries has been further improved. The two-step exercise ECG (Double Master's Test) has been re-interpreted according to the recent World Health Organization recommendations (Blackburn and Rose) for modification of the Minnesota Code. Angina pectoris diagnoses similarly have been updated according to recent WHO recommendations.

More detailed clinical evaluation of cerebrovascular status has been instituted and differential supra-orbital temperature measurements have been added to the examination schedule. Supra-orbital skin is supplied by branches of the internal carotid artery; the potential value of these measurements as a screening device for early detection of cerebrovascular disease will be tested by the follow-up data on these subjects.

The treadmill exercise electrocardiogram has now been performed 436 times on 345 participants and the preliminary results of comparisons with the two-step test results are being strengthened. Using WHO standards, 11 subjects who had negative Master's tests were found to develop definitely positive ECG's on treadmill exercise. Conversely, no subject with a negative treadmill test showed a positive Master's test. The 11 subjects with differing responses

on the two tests will be an especially valuable follow-up group. In addition to these 11 are 23 subjects with negative Master's tests but with "probably positive" treadmill tests. These 2 groups are being examined with respect to the prevalence of coronary risk factors.

The non-invasive assessment of ventricular function in man by computation of systolic time intervals (STI) has been added to the routine examination of the Longitudinal Study Group in the past year. The procedure requires the simultaneous recording of the ECG, the phonocardiogram, and the carotid pulse. In subjects aged 20-93 years, 333 tests have been performed. The correlation of the pre-ejection phase (PEP) and the left ventricular ejection time (LVET) with heart rate noted by Weissler has been remarkably closely confirmed, thus attesting to the standardization and reproducibility of the technique. In addition interesting positive correlations of LVET with systolic BP and of PEP with diastolic BP have been shown by our data; these relations are gratifying in that they make reasonable physiologic sense and further attest to the usefulness of the technique. Age trends in subjects who have no clinical evidence of heart disease are of great interest: the total ejection time and the PEP lengthen with age to reach a peak at age 60; both variables then tend to decline. Since these variables may reflect a worsening of ventricular function with age, the failure to increase after age 60 could be explained on the basis of selective mortality: thus, subjects with greater PEP (poorer function) might have higher mortality rates as they enter later life, so that cross -sectional data may not show further increase in PEP. Longitudinal follow-up will be necessary to confirm or deny this intriguing possibility.

The rat heart continues to offer a difficult but useful preparation for studies on the physiology of aging. Techniques of chronic catheterization of the right atrium and aorta have made it possible to measure BP and cardiac output (CO) in unanaesthetized and relatively unrestrained rats. Arterial BP is measured directly with a strain gauge and CO with the isotopic dilution method (^{86}Rb). In 13 rats aged 24 mo. and 18 rats aged 12 mo., no differences in cardiac function were found. These results in unstressed animals differ from those on isolated heart or heart-lung preparations which should be considered as severely stressed and, indeed, dying preparations. It is now possible in the intact rat to undertake detailed pharmacologic study of control factors in myocardial function as related to age.

A versatile technique which places control of blood glucose concentration at the will of the investigator has been previously described as the "glucose-clamp" method. With its use it has been shown that pancreatic beta cell sensitivity to hyperglycemia declines with age, but that response either to endogenously-released insulin or to exogenously-administered insulin does not appear to decrease with age. There was however a degree of uncertainty in this latter conclusion, since the glucose-clamp technique does not measure the contribution of endogenous glucose production by liver and kidney. Aging might differentially affect the insulin sensitivity of the glucose-producing organs and of the glucose-utilizing organs (primarily skeletal muscle and adipose tissue). The clamp technique was therefore improved by the addition of a glucose-1-^{14}C tracer technique. Hepatic glucose production could thus be computed and an improved estimate of "peripheral" glucose utilization was

available. In the past year 14 tracer studies were performed in young and old male subjects. Basal state glucose production and utilization averaged 1.8 mg glucose per kg body weight per min. and there was no age difference. Insulin was then infused intravenously in such a manner as to create a new steady-state of hyperinsulinemia at a level of 100 μU per ml plasma. There was prompt decrease in hepatic glucose production (R1) and increase in peripheral glucose utilization (R2). By 20 minutes R1 had fallen to only 30% of the basal level and there was no difference between young and old subjects. Furthermore the rate of metabolic recycling of glucose did not differ with age. The conclusion is further strengthened therefore that aging primarily influences beta cell sensitivity rather than tissue sensitivity to insulin.

The glucose-clamp technique provides so much more metabolic information than do the usual clinical glucose tolerance tests, that a series of studies have been done aimed at making it practical to perform these studies on large population groups, and specifically on the Gerontology Research Center's Longitudinal Study Group. The major technical drawback to such an application is the arterial catheterization needed for controlling the arterial glucose concentration. Five "hot-hand" studies were therefore performed, the goal of which were to arterialize the venous blood draining the hand. The technique consisted of placing an electrically-heated glove, thermostatically controlled at 40°C, on the hand and catheterizing a superficial vein draining that hand. Blood flow to the hand was thus greatly increased. A servo-control study was done in the 5 subjects with arterial glucose raised to a new plateau 125 mg per 100 ml above the subject's basal concentration. Arteriovenous glucose differences averaged only 2 mg per 100 ml despite the hyperglycemia and its accompanying hyperinsulinism. In a set of studies in which the hand was not heated, A-V differences averaged 11 mg per 100 ml and the range of individual values was large. Of importance is the fact that insulin A-V differences averaged only 2.6 μU per ml. Thus studies can be done using arterialized venous blood and we anticipate large scale studies in the coming year.

An important collaboration has been established with Dr. Mones Berman of NIAMD for modeling and computer analysis of the kinetics of glucose and insulin in these glucose-clamp studies. These studies have progressed this year to the point that we have established a useful 3 compartment insulin model and there is preliminary evidence that the insulin content in the third pool controls the rate of glucose metabolism.

A series of glucose-clamp studies in prediabetics has been conducted in collaboration with investigators in New York and Boston. There were 7 subjects with normal glucose tolerance tests who were either the offspring of diabetic mothers and fathers, or the identical twin of a diabetic sibling. The major defect discovered in these subjects was a diminution in the rapid insulin response that occurs within the first few minutes after the induction of a new steady state of hyperglycemia.

The chemistry of the renin-angiotensin system has received continued study. A double isotope derivative assay for angiotensin I, previously developed in this laboratory, has in the past year been applied on a routine basis to measurement of renin in human plasma. This is the first entirely chemical

assay for this enzyme and its decapeptide product and represents a significant addition to our ability to quantitate in plasma these important substances, measurements of which are vital for the differential diagnosis of hypertensive diseases. In the past year a variety of physiologic situations (change of posture, salt intake, diuretic administration) have come under study in order to define the normal ranges of renin response. An effort has been initiated to compare by simultaneous assays results by the double isotope technique with bioassay and immunoassay in order to allow evaluation of these widely used but less specific and less precise methods. In our previous report we described the synthesis and preliminary evaluation of a new labeled substrate for renin. This material is the product of coupling a synthetic tridecapeptide renin substrate by its N-terminus to poly-L-glutamic acid, M.W. 50,000, followed by iodination with ^{125}I. We can now report that kinetic data indicate that the synthetic material is in some regards actually a better substrate than the natural protein substrate. The K_M for the synthetic substrate is 0.2×10^{-6} M, which is, remarkably, only one-sixth the K_M of the natural globulin substrate. The V max for the two substrates is approximately the same. The small K_M allows assays with very low concentrations of substrate and in the presence of competing concentrations of globulin substrate. Based on this substrate, a simple assay for renin has been developed which is also extremely sensitive. This should permit the rapid development of renin inhibitors and simplifies efforts to purify human renin. Other similar substrates are being prepared for the assay of renin in human plasma. The polymeric substrate approach seems to have applicability for other enzyme assays, as well.

In the thyroid area detailed studies have been made of certain factors which control the concentration in human plasma of the metabolically active "free" or unbound fraction of thyroid hormone (free T4). Specific ionic effects have been newly described. Chloride ion and phosphate ion have been shown to be concentration dependent inhibitors of T4 binding to plasma proteins. The interaction of these ions, present in ordinary buffers and in plasma, with the effect of dilution, used in equilibrium dialysis to measure free hormone, accounts for a number of previously unexplained observations on the measurement of free T4 in plasma. More importantly, these observations are of significance for understanding the nature and action of naturally occuring inhibitors of T4 binding. An example of such a phenomenon has already appeared in our demonstration in the past year that indoles, a group of compounds known to accumulate in the plasma duirng renal insufficiency, inhibit T4 binding to plasma proteins and may thus account for the elevation of free T4 seen in this clinical state. However, since the binding of indoles to at least one plasma protein, albumin, is known to be competetive with chloride ion, the exact nature of the inhibition by indoles must now be studied in detail with these ionic effects in mind. From our studies in infectious diseases there are indications that other classes of inhibitors of T4 binding may exist. Research into these situations will be guided by our new knowledge of the importance of ion-protein interactions for the binding of thyroid hormone to the plasma proteins.

Serial No. HD-AG3(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Longitudinal Studies of Human Physiology, Biochemistry and Psychology

Previous Serial Number: HD-AG3(c)

Principal Investigators:
N. W. Shock, Director
R. Andres, Clinical Director
A. H. Norris, Coordinator

Other Investigators:
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M. Butler
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Cooperating Units:
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Mrs. Catherine Staneck
Design Methods Section
Data Management Branch
DCRT-NIH
Dr. Samuel W. Greenhouse
Dr. James Schlesselman
Epidemiology and Biometry Branch
OD-NICHD-NIH

Man Years:

Total:	11.15
Professional:	3.00
Other:	8.15

Project Description:

Objectives: This project is designed to: (1) measure various physiological, biochemical and psychological variables repeatedly in the same individuals as they grow older, (2) relate patterns of change in the measurements to theories of aging and (3) establish indices of aging.

Methods Employed: Subjects are volunteers recruited by other participants. They agree to be available for a three-day series of tests every 18 months for the rest of their lives. A given series of tests will be repeated at a five year interval. Measurements of body size and composition, pulmonary function, kidney function, neuromuscular function and cardiovascular function are made. Standardized psychological tests include the Guilford-Zimmerman Temperament Survey, Southern California Tests of Creative-Thinking Abilities (Work Fluency, Associational Fluency I, Expressional Fluency, Ideational Fluency I, Consequences), Benton revised Visual Retention Tests and the vocabulary subtests of the Wechsler Adult Intelligence Scale. Also included are two verbal learning procedures of appropriate difficulty to provide a wide distribution of scores for the longitudinal subjects. Physical examinations and medical histories are performed with check lists to assure uniformity of recording. The classification of electrocardiograms and the diagnosis of angina pectoris are made with systems recommended by the World Health Organization. All electrocardiograms in which myocardial infarction was diagnosed or suspected in the past were reviewed and classified, using the recent WHO modification of the Minnesota Code (Rose and Blackburn). The charts of all subjects with a history of chest pain were reviewed to identify the existence of angina pectoris and to date its onset whenever possible. We have abided closely to the definition of angina expressed by Dr. Goeffrey A. Rose at the London School of Hygiene. Exercise electrocardiograms were examined independently by two physicians, both using criteria of the Minnesota Code. Where discrepancies in applying these criteria arose, the records were reviewed carefully by two other physicians and a best judgment made. Medical findings and pertinent test results are reported to a participant's private physician. Data are kept in restricted files and are summarized by computer methods. The computer based data retrieval system includes: (1) Tabulation card to magnetic tape screening for unacceptable characters, (2) Edit routines which check all fixed form fields for location and reasonableness of entries and (3) Preliminary statistical analyses. The strategy of the study is to maintain 600 active participants. Losses by death, infirmity, and resignation will be replaced by new participants and loss of the youngest subjects by "graduation" into middle age will also be replaced by new volunteers. Secular age changes in this population can thus be followed as well as aging of individual members of the age cohorts.

Major Findings: The measurement program has been continued during the year covered by this report. A total of 897 participants have been tested one time, 692 of these have been tested two times, 563 three times, 448 four times, 345 five times, 247 six times, 144 seven times, 86 eight times, 31 nine times, 15 ten times, and 2 participants have been tested eleven times. Since the beginning of the program, 72 participants have died, 57 have resigned from the program, and 17 have postponed their participation indefi-

nitely. At present, 82 participants have failed to return for tests within three years of their last visit. This leaves an active group of 669 participants. The 220 participants lost to the program are 25% of the group tested one time.

A standardized program has been developed for analysis of the study data. Summaries are provided for two year time cycles. The most recent complete cycle ended June 30, 1969. The current cycle will end June 30, 1971. Several age group summaries are provided: (1) age decade groups, 20-29, 30-39, etc., also 25-34, 35-44, etc., (2) double decades, 20-39, 40-59, etc., and (3) all subjects. Description of the distributions of variables include means, standard deviations, standard errors, selected centile points, and stem and leaf frequency diagrams which provide a graphic display of the distribution and tabulation of each data point in rank order. A longitudinal comparison between cycles can be made by limiting the summaries to subjects who appear in every cycle and using age at entry for all age classifications. An additional longitudinal summary includes all data points of a variable for an individual. Regression of variable on age is calculated and summarized for slope, intercept, confidence limits of slope, etc. by age groups. Lower limits may be placed on the number of data points and/or time period covered by individual regressions to be summarized. This comprehensive summary provides a convenient and appropriate comparison of cross-sectional and longitudinal estimates of change with age in diverse variables in the study.

A detailed classification of participants with respect to cardiovascular disease status has been undertaken. For subjects with histories suggestive of a myocardial infarction, it has in most cases been possible to obtain records of the pertinent hospitalizations or, if they were treated at home, to get corroboration from their private physicians. A more difficult problem has been to acquire adequate information on subjects who have died or failed to return. This information is currently incomplete in 8%, but we expect to reduce this to 2 or 3%.

There have been 788 men who were examined here for the first time prior to July 1, 1969. Of these, a total of 115 (15%) have had demonstrable coronary heart disease as evidenced by angina pectoris, myocardial infarction, sudden death of probable coronary origin, or an unequivocally positive exercise electrocardiogram (Master's test). Of the 788, 57 (7.2%) have experienced angina pectoris, 61 (7.7%) have suffered myocardial infarctions prior to July 1, 1969, and 28 (3.5%) have had positive exercise electrocardiograms (ST segment depression of 1mm or more). An additional 39 (5.0%) have shown "borderline, probably positive" exercise electrocardiograms (ST segment depression of 0.5-0.9mm) as their only indication of coronary heart disease. If these are included, the total of the subjects with evidence of coronary heart disease rises to 154 (20%).

Of the 57 subjects with angina, 23 also have had a myocardial infarction. Angina is known to have preceded the infarction in 6. Twenty-seven subjects incurred an infarction at some time after their initial participation in the study. Of these, 17 were preceded by a history of angina, a signif-

icantly abnormal resting electrocardiogram or a positive exercise electrocardiogram.

Significance to Bio-Medical Research and the Program of the Institute: A major goal of the Longitudinal Program is a deeper understanding of age-related changes in the different organ systems, and their interrelationships. In this regard, the identification of coronary artery disease serves to characterize an age-related disease in the cardiovascular system. Numerous studies elsewhere have demonstrated that heart disease occurs with increased frequency in those who are hypertensive, inactive, obese or diabetic and in those who smoke cigarettes or have elevated blood lipid levels. The potential contribution of our Longitudinal Study to this important public health problem lies in the intensity of study of a number of these variables in this study.

Collection of this body of data will place us in a position to test theories of aging with the human population of the longitudinal studies. Results of comparisons between old and young individuals are presently available, but these do not permit us to evaluate theories of aging adequately. Our ability to do so will depend in part upon a comparison of the integrated medical, physiological, psychological and biochemical profiles of the individual participant with various subsequent outcomes which we may be in a position to monitor in the same individual. Outcomes such as length of life, occurrence of disease, rate of change in a functional parameter, among other things, will be sought. Some theories assume that a few cells are lost during each unit of time so that in an individual or in an individual organ system changes of a gradual nature would be found. These theories are consistent with the summation of a series of distributed minor accidents (such as radiation hits) if only a few cells are involved each time. Other theories would relate large changes in function with the occurrence of a disease episode, or major accident resulting in a substantial loss of cells. These theories are consistent with abrupt changes in functional levels which would be maintained until another major accident results in a new level. In addition, these two widely different types of theories could be found in combination with sudden as well as slow changes operating simultaneously.

Proposed Course of Project: Measurements and examinations of male participants will be continued. A similar program for women should be initiated. This would permit a search for the factors which lead to the "edge" in longevity which women have over men. Analysis and evaluation of data in the file will continue.

Honors and Awards:

Dr. Shock is President of the International Association of Gerontology.

Publications: None

Serial No. HD-AG4(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Age Changes in Human Performance

Previous Serial Number: HD-AG4(c)

Principal Investigator: A. H. Norris

Other Investigator: N. W. Shock

Cooperation Units: Baltimore City Hospitals
Dr. A. T. Welford
Department of Psychology
The University of Adelaide
Adelaide, South Australia

Man Years:

Total:	3.00
Professional:	0.90
Other:	2.10

Project Description:

Objectives: This project is designed to study the effects of aging on: (a) the physiologic responses to exercise, (b) the rate of recovery of physiologic equilibrium after exercise, (c) muscular efficiency and (d) work output and fatigue. In addition, the factors responsible for limitations of performance observed in older people will be evaluated.

Methods Employed: Measured amounts of physical work are obtained in subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood pressure, and electrocardiogram are made before, during and after standardized amounts of exercise. In addition, the volume of the lungs and functional capacities of the pulmonary system are evaluated. Other studies include measurements of speed of nerve conduction, reflex latency and muscle action potentials.

Major Findings: The speed and accuracy of movement during a "tapping" test have been analyzed for 300 subjects in addition to 325 subjects previously reported. Longitudinal analysis of age changes have provided a

different interpretation than the one reported last year (1969-1970). At that time, it was felt that faster motor control declined more with age than slower visual control of movement. The current summary shows an equal decline in these components of control of movement. Moreover, the accuracy of absolute positioning on target was felt to decrease with age. The current summary shows no age difference in this accuracy. The test required subjects to tap alternately on two targets drawn on paper. Width of target and distance between targets were considered separately, the relation between time per movement and amplitude of movement (distance between targets) was a slope of approximately 10 bits per second while the relationship of time per movement and accuracy on targets was a slope of approximately 5 bits per second. This suggests two control processes: a faster one concerned with distance-covering and a slower one of "homing" onto the target. It seems reasonable to regard the former as essentially motor control shown in relatively pure form in ballistic movements aimed at achieving a given amplitude but not at a definite target, and the latter implying an additional process of visual control. Thus: movement time = a log A' + 1/W; where a and b are slope constants for amplitudes and targets respectively. Since ballistic movements show substantial accuracy without current visual control, we have added an estimate of this accuracy. Thus: movement time = a log A' - b log W_i' + (b - a) log W_o'; where W_o' is the scatter of shots that would be observed with ballistic movements of amplitude A', and W_i' is the scatter observed with any particular target under consideration. The latter equation was used to summarize the results for more than 600 longitudinal studies participants. Of these, 265 had four or more tests performed a year or more apart. Individual regression statistics for these subjects were summarized in the-year age groups (25-34, 35-44, etc) Both motor control performance and visual control performance declined by 30% between the younger and older age groups. Slope a and its reciprocal in bits per second for the variation of amplitude with accuracy held constant, declined from 10 bits per second in 25 to 34 year old subjects to 7 bits per second in 75 to 84 year old subjects. Slope b and its reciprocal in bits per second for variation of accuracy with amplitude held constant, declined from 5 bits per second in 25 to 34 year old subjects to 3.5 bits per second in 75 to 84 year old subjects. Subjects younger than 55 years of age tended to improve their motor control performance over time (from test to test) while subjects over 55 years of age showed either no improvement or a decline in motor control performance. Only the youngest age group (25-34 years) showed an improvement in visual control performance over time (test to test). Subjects over 45 years old tended to decline in visual control performance over time with the greatest declines in subjects over 65 years old. The accuracy of absolute positioning indicated by W_o' was 24 mm for subjects between 30 and 84 years of age. This consistency of W_o' is associated with appreciable declines with age both in motor control performance and in visual control performance. These declines suggest that both rapid motor performance and slower visual (descisional) performance may depend on a common support mechanism in the central nervous system which is subject to deleterious age changes.

Significance to Bio-Medical Research and the Program of the Institute: The decline of the ability of some older people to perform their day-to-day

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activities and to engage in pursuits which contribute to the economic and social strength of our society represents a national loss. Identification of the physiological, medical and social correlates of high levels of physical strength and psycho-motor performance in middle and old age, as well as declines in these abilities, should lead to techniques designed to reduce the rate of decline in performance capacities with age.

Proposed Course of Project: Appraisal of participants in the longitudinal studies will be based on measurements of muscle strength and maximum power generating ability during arm exercise. Cardiovascular, ventilatory and metabolic responses to standardized arm ergometer exercise and monitored treadmill exercise will be used to classify participants into fitness categories. Measurements of lung volumes and uniformity of pulmonary ventilation will be made to characterize the respiratory competence of the longitudinal studies participants and to evaluate the benefits of the cessation of smoking in this group.

Honors and Awards: None

Publications: None

Serial No. HD-AG5(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Age relationship of body composition, nutrition and physical activity

Previous Serial Number: HD-AG5(c)

Principal Investigator: A. H. Norris

Other Investigators: R. Andres
N. W. Shock
M. Butler

Cooperating Units: Baltimore City Hospitals
Professor George Vose
Research Institute
Texas Women's University
Denton, Dexas
Dr. Harald Schraer
Department of Biophysics
Pennsylvania State University
University Park, Pennsylvania
Dr. Robert B. McGandy
Department of Nutrition
Harvard School of Public Health
Boston, Massachusetts

Man Years:

Total:	3.05
Professional:	1.30
Other:	1.75

Project Description:

Objectives: This project is designed to describe age differences in body composition, nutrition and physical activity made concurrently in individual subjects, to examine the relationship of these age differences, and to compare them to selected physiological and biochemical characteristics of the subjects.

Methods Employed: Height and weight are obtained by the usual anthropometric methods. The volume of the body is measured by its displacement

of helium in a closed chamber. The density of the body is calculated from these measures after correction for total skeletal mass estimated from skinfold thickness and roentgenographic techniques. Estimates of indices of lean body mass include: (1) basal metabolic rate determinations made with standard open circuit methods, (2) twenty-four hour urinary excretion of creatinine and (3) total body water and extracellular water determinations made from the distribution curves of injected antipyrine and sodium thiocyanate. Nutrient intakes and eating habits are estimated from diet diaries and interviews. Physical activity levels are estimated from interviews and self-administered questionnaires. This study is part of the continuing Longitudinal Study. The studies are carried out on people of different ages (cross sectional) and on the same people as they become old (longitudinal).

Major Findings: Summaries of serial measurements of body composition have been made for individual participants in the Baltimore Longitudinal Study. These measurements of change in individuals were summarized by ten year age groups. They provide a more sensitive estimate of the relation between age and body composition than do age group comparisons where one-time measurements for individuals are used (cross-sectional estimates). While the cross-sectional estimates imply a decrease of 0.1 cm per year for height, summaries of serial measurements (longitudinal estimates) show that the rate of change for men 25-54 years old is 0.06 and 0.2 cm per year for men 55-84 years old. A similar age pattern is seen in weight. The cross-sectional weight estimates show no change in subjects 25-54 years old and decrease of 0.2 kg per year in subjects 55-84 years old. However, longitudinal estimates of change show that these men 25-54 years of age are gaining weight at about 0.3 kg per year while 65-84 year old subjects are losing weight at a rate of 0.4 kg per year. Subjects 55-64 years old are in an intermediate age and weight change position. They lose at 0.15 kg per year. This pattern of weight gain and loss was matched by the longitudinal finding of first increase then decrease in serum cholesterol concentration in this population.

Other body composition indices were also reviewed in terms of the serial measurement summaries. Skinfold thickness measurements showed no age differences by cross-sectional estimates. Only left and right abdominal thickness showed lower values in subjects over 65 years old. Longitudinal estimates, on the other hand, showed a clear pattern of decrease of thickness in chest and abdominal sites and no change in thickness in upper arm (over triceps b.) and back sites for all age decade groups between 25 and 84 years of age. The chin thickness matched the loss in chest and abdominal thickness in the age decade groups between 35 and 84 years of age. Moreover, skinfold thickness at the right and left chest sites decreased three times as fast in subjects 55 to 84 years old as in subjects 25-54 years old.

The high rate of loss of fat from the chest and abdominal sites in subjects 25-54 years old does not agree with the weight gain during those years. A redistribution of fat to unmeasured sites might explain the result but the cause is not clear. It is possible that physical activity (such as jogging) could have caused the redistribution of fat or fat loss associated with gain

in lean body weight. While these are unlikely trends for U.S. men, they may be tenable for this selected group of participants.

Significance to Bio-Medical Research and the Program of the Institute: Many diseases of the later years as well as aging changes in organ systems which are not clearly identifiable as "disease" are thought to be related to such life-long factors as diet, activity levels, and body composition. By assessing these variables periodically during a significant fraction of the total life span, it should be possible to determine the relative contribution of these factors to longevity and, more importantly, to the maintenence of health and vigor into the later years of life. These variables cannot be accurately estimated by distant recall technique (retrospective history) and the prospective longitudinal approach is a necessary requirement for the collection of reliable data.

Proposed Course: All studies are to be performed on the Longitudinal Study participants according to schedule. Summaries of weight and fat indices will be compared with estimates of lean body mass, muscle mass and bone density changes as well as activity levels and food intake.

Honors and Awards: None

Publications: None

Serial No. HD-AG1(c)7
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Marital, Sexual, and Social Factors in Aging

Previous Serial Number: HD-AG1 (c) 7

Principal Investigator: C. Martin

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.00
Professional:	1.00
Other:	0.00

Project Description:

Objectives: Major objectives are: (1) to describe how various indicators of marital adjustment, sexual reactivity, and sexual performance are related to generation and age; (2) to determine whether individual differences in sexual functioning may be related to some aspect of health, physiologic state or psychologic trait; and (3) to discover whether attributes of social, marital or sexual experience may be associated with the onset of disease or with longevity.

Methods Employed: Data collection has been conducted by questionnaires and personal interview. On predetermined visits to the Gerontology Research Center, study participants are asked to complete one particular questionnaire (Your Activities and Attitudes, Burgess, Cavan, and Havinghurst) which requests various kinds of demographic and attitudinal information. While the questionnaire was initially designed to provide scores pertaining to social adjustment, many items on the questionnaire allow participants to be classified in numerous ways for purposes of prospective investigation.

In addition, each subject in the study is asked to take part in an interview concerning his history of marriage and sexual activity. In making the request, the objectives of the study are carefully described, confidence is assured, and the voluntary nature of the interview is emphasized. Thus far, 6 refusals were encountered in response to 520 requests for such an interview.

Major Findings: In a search for factors related to the onset of erectile

impotence, 67 subjects were identified as married males aged 40-79 who reported recurring failure to obtain and/or sustain erection in their coital relationships. To these were matched 67 married subjects of the same age (control group) who reported continuing coital activity and full potency. The two groups were then compared on some 48 attributes of sexual, marital, and other social experience.

No significant differences between the groups were found with reference to:

1. Educational attainment, religious background, farm residence in early life, parental social status, or parental broken home.
2. Number of years married, times married, age at first marriage.
3. Number of coital partners prior to 40 years of age.
4. Quantity of coital or ejaculatory activity between 20 and 40 years of age.
5. Episodes of coital abstinence after the onset of coital relations.
6. Circumcision status or history of prepubescent sex play.
7. Customary or maximum frequency of coitus in early marriage.
8. Coitus with prostitutes, adult homosexual activity, or venereal infection.
9. Present state of responsiveness to heterosexual stimuli.
10. Occurrence of sexual dreams or masturbatory activity.

Significantly more case than control subjects reported a continuing problem with premature ejaculation (27% vs. 9%); orgasm half the time or less on the part of wives in coitus (53% vs. 36%); and poor marital adjustment in recent years (15% vs. 3%). These factors together appear to constitute a pattern indicative of imparied marital and sexual relationships which had more often occurred in case than control marriages. Thus, present findings lend some support to the hypothesis that psychologic factors are etiologically involved.

On the other hand while the long-term effects of premature ejaculation may plausibly account for poor orgasmic response among wives and perhaps poor marital adjustment, the majority of case marriages appear to lack this pattern of impaired sexual relationships. Overall, the evidence suggests that other, as yet unidentified factors which may or may not be psychological, are likely involved.

Significance to Bio-Medical Research: While the study of marital and sexual factors in disease and longevity has been a neglected subject, the present study of etiologic factors in erectile impotence more carefully defines the type and extent of erectile disturbance than is usually the case, it is almost alone in utilizing a control group of known potency, and uniquely, none of the subjects involved were self-selected for sexual disorder. In a sense, the study will be among the first to explore a variety of variables based upon systematically obtained data.

Proposed Course: Plans are to assess the significance of the following factors as they may be associated with various medical and physiologic measures:

1. frequency of attendance at religious services (recent evidence shows an unexplained lower incidence of coronary disease in church goers)
2. number of changes of marital status
3. number of coital partners
4. quantity of sexual experience between 20 and 40 years of age.

Honors and Awards: None

Publications: None

Serial No. HD-CP4(9)(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Significance of heterozygous deficiency of serum alpha-1-antitrypsin

Previous Serial Number: HD-CP4(9)(c)

Principal Investigator: Frank N. Turner

Other Investigator: Arthur H. Norris

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	0.70
Professional:	0.50
Other:	0.20

Project Description:

Objectives: The objective of this project is to characterize each participant in the longitudinal study with respect to his plasma alpha-1-antitrypsin level. The relationship of pulmonary performance to serum trypsin-inhibition capacity will be evaluated in order to test the hypothesis that deficiencies in this enzyme may be related to the development of chronic lung disease.

Methods Employed: The method of Eriksson (Acta Medica Scandinavica, Vol. 176, Supp. 432, pp. 6-12) was used in preliminary work last year. During the past year Eriksson's method has been modified for use with the Technicon Auto-analyzer. Detailed pulmonary function tests are performed on each participant in the Longitudinal Study on each visit, as noted in previous reports.

Major Findings: It was confirmed that the method chosen was reliable when performed manually, albeit tedious. Since it is planned to analyze antitrypsin levels in each participant on previously obtained plasma stored in the lyophilized state, it was necessary to study enzyme stability in stored samples.

Evaluation of both reagent and sample stability was done over a 4 month period. It was found that the reagents were stable for periods up to 4 weeks if kept refrigerated. Trypsin was found to give a consistent optical density, and both serum and soybean inhibition in appropriate concentrations

gave reproducible degrees of inhibition.

Samples of plasma subjected to repeated freezing and thawing resulted in lost trypsin inhibiting capacity, but samples kept frozen retained their activity for up to 4 months.

Lyophilization of plasma did not destroy activity, and activity was stable for at least four months. The trypsin inhibiting capacity of plasma was found to be equivalent to that of serum.

The necessity for mass analysis of many hundreds of stored plasma samples made it desirable to attempt to automate the analytical procedure. We were able to design a system which gives an excellent sensitivity range, reproducibility, and clear separation of sample peaks (i.e. without "carryover artefact").

Analysis of these samples has just begun. We will analyze at least one sample on each individual and will also do repeat studies on a selected group of subjects who have had repeated visits to the Center. (These might be called "retrospective longitudinal analyses" since they will all be done on lyophilized samples.)

Significance to Bio-Medical Research and the Program of the Institute: The homozygous deficiency of alpha-1-antitrypsin, although rare, has been clearly associated with early-onset emphysema. The demonstration of a less pronounced but definite association between the more common heterozygous deficiency state and emphysema would allow ready identification of high risk patients. These patients could be used in the evaluation of preventative programs and therapeutic regimens. They could also be studied longitudinally in an effort to deduce the pathogenesis of obstructive lung disease, a major source of morbidity in the older age population.

Proposed Course: The plasma samples will be analyzed for trypsin inhibiting capacity. The population will be characterized with respect to trypsin inhibiting capacity. The trypsin inhibiting capacity will be examined as an independent variable in the evaluation of the longitudinal program pulmonary function data.

Honors and Awards: None

Publications: None

Serial No. HD-CP2(1)(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Identification and characterization of cerebrovascular disease in Longitudinal Participants and correlations with other parameters of physical and psychological function.

Previous Serial Number: New Project

Principal Investigators: C. Paul Scott

Other Investigators: Arthur H. Norris
Reubin Andres

Cooperating Units: David Reissler
Lewis H. Kuller
Department of Chronic Diseases
The Johns Hopkins University
School of Hygiene and Public Health
Baltimore, Maryland

Man Years:

Total:	1.00
Professional:	1.00
Other:	0.00

Project Description:

Objectives: (1) To establish the prevalence and incidences rates of cerebrovascular disease (CVD) among participants in the longitudinal study. (2) To evaluate and utilize objective tests of presence, classification, and degree of CVD. (3) To correlate the presence of CVD with other measures of physiological function in longitudinal participants. (4) To provide a large population of generally healthy individuals to validate certain measures described below (thermometry, tonography and the transient ischemic attack questionnaire) and help establish normal values for these tests, previously used on rather small and ill populations.

Methods Employed: Several noninvasive, nontraumatic objective measures are being utilized to identify subjects with definite or possible evidence of cerebrovascular disease (CVD): (1) Examining physicians are alerted to CVD diagnoses by inclusion of a special form; this form, the patient, and his entire record are reviewed as necessary. (2) Careful follow-up of private physician's and hospital records is made. (3) Blood pressure and carotid

pulses and bruits are examined systematically. (4) Direct thermometry of the medial supraorbital area of the forehead, as described by Austin and Asjid, is carried out under controlled conditions. (5) A questionnaire to ascertain a history of transient ischemic attacks (TIA), recently developed by researchers at the Johns Hopkins School of Public Health, is administered by a technician and scored according to an arbitrary standardized grading system. (6) Schoitz tonography, as described by Knox, will be utilized to measure pulse pressure. Like thermometry, this method is conceived as indicating internal corotid patency. Other procedures, such as (7) radio cine-scan cerebral transit studies, will be used where indicated by results of other tests.

Major Findings: In the initial retrospective review of all 800 longitudinal participants, twenty participants were identified with definite evidence of CVD--their years of birth range from 1869 to 1919 (median 1889); the age at onset of CVD ranges from 42 to 92 (median 72); their diagnoses include cerebral thrombisis 55%, cerebral embolism 10%, TIA 20%, and cerebral atherosclerosis 15%; six have died at ages from 79 to 99. Ten others with probable CVD were identified. To this date, 100 participants have been screened by the techniques described in the methods section. Nineteen of the 100 were identified by examining physicians as having evidence of symptomatic CVD, possible or probable; four of the nineteen were previously known to have CVD by the initial retrospective chart review. The remaining fifteen will be reviewed to allow separation of false-negatives from the mass of participants without CVD.

Significance to Bio-Medical Research and the Program of the Institute: Cerebrovascular disease is a major cause of death and morbidity in aging, and means are now being devised and tested to screen normal populations for precursors of the disease. The knowledge gained with better characterization of those individuals in the longitudinal study may lead to improved preventive measures and treatment of this and other serios illnesses.

Proposed Course of Project: The presence, absence, and/or degree of CVD will be correlated with several tests available, on a cross-sectional as well as longitudinal basis. Among the tests to be used will be psychological measures such as learning and problem solving; physical and diagnostic categories such as blood pressure, atherosclerotic heart disease, serum lipids, impaired carbohydrate tolerance and kidney function; and the Cornell Medical Index Health Questionnaire. It is expected that in 18 months the entire active study population will be surveyed, allowing meaningful characterizations and correlations to be made on those participants with CVD.

Honors and Awards: None

Publications: None

Serial No. HD-CP2(1)(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Identification and characterization of cerebrovascular disease in Longitudinal Participants and correlations with other parameters of physical and psychological function.

Previous Serial Number: New Project

Principal Investigators: C. Paul Scott

Other Investigators: Arthur H. Norris
Reubin Andres

Cooperating Units: David Reissler
Lewis H. Kuller
Department of Chronic Diseases
The Johns Hopkins University
School of Hygiene and Public Health
Baltimore, Maryland

Man Years:

Total:	1.00
Professional:	1.00
Other:	0.00

Project Description:

Objectives: (1) To establish the prevalence and incidences rates of cerebrovascular disease (CVD) among participants in the longitudinal study. (2) To evaluate and utilize objective tests of presence, classification, and degree of CVD. (3) To correlate the presence of CVD with other measures of physiological function in longitudinal participants. (4) To provide a large population of generally healthy individuals to validate certain measures described below (thermometry, tonography and the transient ischemic attack questionnaire) and help establish normal values for these tests, previously used on rather small and ill populations.

Methods Employed: Several noninvasive, nontraumatic objective measures are being utilized to identify subjects with definite or possible evidence of cerebrovascular disease (CVD): (1) Examining physicians are alerted to CVD diagnoses by inclusion of a special form; this form, the patient, and his entire record are reviewed as necessary. (2) Careful follow-up of private physician's and hospital records is made. (3) Blood pressure and carotid

tests are performed and interpreted according to the W.H.O. recommended modification of the Minnesota Code.

All longitudinal subjects who (1) have not had a documented myocardial infarcion, (2) are not taking digitalis preparations, quinidine, coronary vasodilators, or other cardiotonic or cardiodepressant drugs, and (3) can physically perform treadmill exercise are selected for stress electrocardiography testing.

Light-weight fluid conduction electrodes are applied after appropriate skin preparation, and are used to record the standard limb leads and the CR leads. Resting, in-exercise and post-exercise EKG's are constantly monitored by oscilloscope and recorded on magnetic tape.

Subjects are asked to walk at a comfortable gait on a motor-driven treadmill, programmed to accelerate from 1.0 to 3.5 M.P.H. and from 0° to 9° elevation (15% grade) in four steps, at the end of each of the first four minutes. Subjects continue exercising until (a) fatigue, (b) dyspnea, (c) pain or (d) other distress prevents them from continuing or until (e) ischemic ST segment depression or (f) multiple PVC's appear on the ECG, or (g) until heart rate reaches a plateau as determined by cardiotachometer.

Major Findings: 436 electrocardiographic stress tests have been performed in 345 longitudinal participants. The relationship between the Master's two-step test and the programmed treadmill exercise test is shown in the following table:

TREADMILL STRESS TEST

MASTER'S TEST	POSITIVE	BORDERLINE		NEGATIVE	TOTALS
		PROB. POSITIVE	PROB. NEGATIVE		
POSITIVE	5	0	1	0	6
BORDERLINE, PROB. POSITIVE	7	1	3	3	14
BORDERLINE, PROB. NEGATIVE	8	1	33	34	76
NEGATIVE	11	3	24	302	340
TOTALS	31	5	61	339	436

It is clear that more positive responses are obtained with the programmed treadmill regimen. Presumably this represents increased sensitivity in the detection of early coronary artery disease, but long-term follow-up of these subjects is required in order to rule out the possibility of decreased specificity (false positives) on the stress test. 88 subjects have had at least two stress tests and the results on the paired tests are quite similar, with no trend in either direction as yet. There have been no untoward effects of testing in 26 months.

Significance to Bio-Medical Research and the Program of the Institute: Stress electrocardiography has been shown to be a sensitive predictor of ischemic heart disease. The opportunity to correlate data obtained using this test with data from a large number of additional known and suspected predictors of coronary heart disease, and to do so longitudinally on a stable population should greatly increase understanding of the natural history of coronary heart disease.

Proposed Course of Project: The stress EKG program is now incorporated into the longitudinal aging program. Its value as a predictor of overt coronary heart disease is currently being evaluated in a major review of coronary risk factors currently being undertaken at the Gerontology Research Center.

Honors and Awards: None

Publications: None

Serial No. HD-CP6(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Cardiovascular Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Non-invasive evaluation of ventricular function in man by measurement of systolic time intervals

Previous Serial Number: HD-CP 6 (c)

Principal Investigators: David J. Shaw
Donald A. Rothbaum

Other Investigator: N. W. Shock

Cooperating Units: Baltimore City Hospitals

Man Years:
Total: .40
Professional: .30
Other: .10

Project Description:

Objectives: Several investigators have established the validity of measuring systolic time intervals (STI's) by non-invasive means as measures of ventricular function in man. Work from other centers has indicated that there may be age differences in some intervals suggesting a decline in ventricular function with age in man. This study is being undertaken with the purposes of: (1) confirming differences in systolic time intervals among different age groups in cross-sectional studies; (2) determining changes in STI's in individual participants in the longitudinal study; (3) seeking a mechanism for any observed age changes by pharmacologic and/or physiologic intervention, and (4) establishing a sensitive and objective clinical indicator of deteriorating ventricular function in the subjects participating in the longitudinal aging program of the Gerontology Research Center.

Methods Employed: Systolic time intervals are measured in man by simultaneous high speed recording of a single lead of the electrocardiogram, the phonocardiogram, and the externally recorded carotid pulse, on an oscillographic recorder. Specific intervals measured include: (1) R - R interval, (2) QRS duration, (3) QS_2, the total duration of systole, from the onset of the QRS complex to the first high frequency aortic component of the second heart sound, (4) LVET, the left ventricular ejection time, measured from the onset of the upstroke of the carotid pulse to the nadir of the

dicrotic notch. From these four intervals are calculated three additional parameters: (1) heart rate (reciprocal of R - R interval); (2) the pre-ejection period (PEP), PEP = QS_2 - LVET; and (3) the ratio of PEP/LVET. In addition a detailed current smoking, drug use and activity history is obtained from each subject and blood pressure is measured by cuff, immediately after each test. An estimate of average daily activity in calories is derived from a self-administered detailed activity questionaire.

Work done by Weissler and others has demonstrated that pre-ejection period increased, LVET decreases, and the ratio PEP/LVET increases with deteriorating ventricular function as measured by independent methods at cardiac catheterization.

Major Findings: Systolic time intervals have been recorded at rest in 333 participants in the longitudinal aging program of the Gerontology Research Center, ranging in age from 20 to 93 years. Of these, 12 had heart disease with congestive heart failure, 70 had compensated heart disease and 40 had hypertension only. The remaining 211 subjects were used to generate normal regression data for the longitudinal study group.

Weissler and others have demonstrated clearly the negative linear correlation between heart rate and each of QS_2, LVET and PEP. We found highly significant ($P<.0001$) correlations for each of these pairs. The regression equations, given below, are almost identical to those found by Weissler.

$QS_2 = (-2.25 \times \text{HEART RATE}) + 547.0$
LVET = $(-1.73 \times \text{HEART RATE}) + 411.0$
PEP = $(-.54 \times \text{HEART RATE}) + 137.0$

The intercepts of these three equations are called respectively the QS_2 Index (QS_2I), ejection time index (ETI), and pre-ejection period index (PEPI). The above indices compare with Weissler's values of $QS_2I = 546$, LVETI = 413, and PEPI = 131. Some additional correlations of interest were noted which have not been previously described, and are shown below:

$QS_2I = (.235 \times \text{SBP}) + 520.0$, $P<.025$
LVETI = $(.210 \times \text{SBP}) + 386.0$, $P<.025$
PEP = $(.310 \times \text{DBP}) + 79.0$, $P<.025$

Possible explanations for the above rather modest correlations follow: The prolongation of ejection time, and hence total systole with increasing blood pressure might be predicted from the higher ultimate level to which blood pressure must be driven. The prolongation of pre-ejection period with increasing diastolic pressure (afterload) is predictable from the consideration that the final blood pressure level reached before the aortic valve opens is a determinant of the duration of the pre-ejection period.

Finally, a number of observations have been made in this cross-sectional study concerning age differences. In our population, as has been noted in many others, systolic blood pressure increased, and activity decreased with age (for both, $P<.001$). When the entire population over the age span 20-90 years was examined, no significant correlations could be demonstrated

for any of the systolic time intervals, or their indices with age. However, when the PEP or PEPI was plotted versus age in a scatter plot, an interesting pattern emerged: up to age 60 (N=143) PEP and PEPI both increased slightly, but highly significant ($P<.001$) with age, at a rate of about 4 msec./decade. Beyond age 60, however, variance increased somewhat and the parameters tended to decline somewhat from their peak at age 60. The same observation of correlation of the interval with age for subjects under 60 was found for both the QS_2 and QS_2I, ($P<.01$). When the systolic time intervals were examined in our population with heart disease or hypertension, there were small increases in both PEP and PEPI and decreases in LVET and LVETI, but these were not statistically significant, reflecting primarily the generally excellent state of health of the participants in the program, and the rigid criteria which were used to exclude these subjects from our "normal" population.

Significance to Bio-Medical Research and the Program of the Institute: The measurement of systolic time intervals provides a convenient non-invasive method of measuring ventricular function in a large number of individuals. It is unclear at the present time, however, just how sensitive these indices are. The opportunity to make repeated measurements longitudinally in the same population, and to correlate the measurements with other laboratory and clinical indices of cardiac function will further define the sensitivity of the intervals.

The observation of increasing pre-ejection period up to about age 60 with a slight decrease thereafter is most intriguing, and suggests perhaps an analogy with age changes in blood pressure measured in large populations. There may be two (or more) populations, one of whom has an increasing pre-ejection period, and hence deteriorating ventricular function with age, and hence is at increased risk of dying young, while a second population whose indices do not change with age and hence whose heart remains compensated, survives into the latter decades. This hypothesis must be tested by the use of systolic time interval measurements on a longitudinal basis in a stable population such as that in the longitudinal aging study of the Gerontology Research Center.

Proposed Course of Project: Initial measurements will be made in those participants in the Longitudinal Aging Program who have not yet had them, and repeat measurements will be obtained as participants return. In addition use of a stress such as sustained isometric handgrip to attempt to increase the sensitivity of the test is being evaluated.

Honors and Awards: None

Publications: None

Serial No. HD-CP10
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Cardiovascular Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Age changes of hemodynamic variables in intact, unanesthetized rats

Previous Serial Number: HD-CP 10

Principal Investigators: David J. Shaw
Donald A. Rothbaum

Other Investigator: N. W. Shock

Cooperating Units: Baltimore City Hospitals

Man Years:
Total: 1.80
Professional: 1.60
Other: .20

Project Description:

Objectives: In order to study mechanisms of hemodynamic age changes known to occur in man, procedures must be used which are not applicable to humans. Hence it is necessary as a first step to determine whether similar age changes occur in experimental animals.

This project has been undertaken, then, in an effort to: (1) describe hemodynamic changes which occur with age as measured in intact, unanesthetized rats, and (2) explore, where feasible in this preparation, mechanisms for changes observed.

Methods Employed: Under pentobarbital anesthesia PE 50 polyethylene catheters are placed in the left common carotid artery and right external jugular vein of 12 and 24 month old male rats, and the rats are allowed to recover. Phasic and mean arterial blood pressure are measured by means of a Statham strain gauge transducer, cardiac output is measured by an indicator dilution technique using the ^{86}Rb isotopic method described by Sapirstein, and heart rate is measured from the phasic blood pressure recording. From the indices heart rate, mean blood pressure, cardiac output and body weight are derived cardiac index, stroke volume, stroke index, peripheral vascular resistance, left ventricular minute work, LV work index, stroke work and stroke work index.

Animals are studied on postoperative days 7 and 9. The animals are placed in small, non-confining plexiglass cages, and lengths of PE 50 polyethylene tubing are attached to the arterial and venous catheters. In this way, the animals can be studied without further handling, and are relatively unstressed. On the 9th postoperative day, immediately following duplicate determinations of resting cardiac output and blood pressure, propranolol is administered I.V. and duplicate cardiac output and blood pressure measurements are made 30 and 45 minutes after administration of the drug.

Major Findings: Resting cardiac outputs and blood pressures have been measured in eighteen 12 month and thirteen 24 month old Wistar rats from the animal colony of the Gerontology Research Center. The indices reported agree closely with those same indices reported in the literature for unanesthetized rats using both this and other methods of measuring cardiac output. None of the indices differed significantly in the two age groups.

The same indices were measured after administration of propranolol in twelve 12 month and ten 24 month old animals. The mean response of all parameters to the drug using each animal as its own control was virtually identical in the young and old groups, and so the results of response to propranolol are summarized for the 22 animals which received propranolol, taken as a whole. Significant changes were found in heart rate (-15%), diastolic blood pressure (-8%), cardiac index (-15%), peripheral vascular resistance (+13%), and left ventricular work index (-17.5%) following propanolol administration. There was no significant changes in stroke index. Thus, in the rat, propranolol exerts its effect on cardiac output purely by a negative chronotropic response, without affecting stroke output at all. In addition, the increase in peripheral resistance is accomplished solely through the decrease in cardiac output, as there is actually a slight decrease in mean arterial pressure.

Significance to Bio-Medical Research and the Program of the Institute: In a model free of arteriosclerotic heart disease, in which physiologic control mechanisms are intact, no-age related changes could be demonstrated at rest or following beta sympathetic blockade (which did produce appreciable changes in cardiovascular performance in both young and old animals). Thus any functional changes in cardiac performance due to aging of the heart which may exist would appear to require more rigorous stress for their demonstration in this model.

Proposed Course of Project: The preparation described is currently being used for an evaluation of the response of young and old animals to an acute pressor stress with intravenous infusion of the alpha adrenergic stimulator, phenylephrine. Following this, it is anticipated that the response of animals to swimming with and without propranolol, and to alpha adrenergic blockade, cholinergic blockade and beta and cholinergic stimulation will be investigated in an attempt to map any age differences in control mechanisms in the cardiovascular system.

Honors and Awards: None
Publications: None

Serial No. HD-AG10(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effect of age on carbohydrate metabolism

Previous Serial Number: HD-AG 10 (c)

Principal Investigators: John Liljenquist
Paul Insel
Jordan Tobin
Reubin Andres

Other Investigators: Robert Sherwin
Robert Gregerman
Philip Thorne

Cooperating Units: Mones Berman
Mathematical Research Branch
NIAMD

Isaac Hantman
Donald Deutsche
Computer Systems Laboratory
Division of Computer Research & Technology

Man Years:
Total: 4.75
Professional: 2.30
Other: 2.45

Project Description:

Objectives: This project is part of the long-term prospective study of human aging, the Longitudinal Study of the Gerontology Research Center. The complex interplay of aging, carbohydrate metabolism, and diabetes mellitus offers a prototype of one of the serious challenges to clinical gerontology, the differentiation of normal aging changes from specific disease states. The objectives of this project are: (1) to describe cross-sectional changes during the entire adult span of life in performance on the commonly-used clinical tests for the diagnosis of diabetes, (2) to provide age-adjusted standards of normality for these tests, (3) to define the relative sensitivity and specificity of these tests, (4) to establish the role of certain experimental variables which influence performance on these tests, (5) to

investigate the mechanisms underlying the age changes, and (6) to evaluate the significance of the decline in tolerance [a] by noting the influence of poor performance on mortality and [b] by testing for correlations between performance and certain other variables known to be clearly associated with the overt diabetic state.

Methods Employed: Subjects are primarily those in the Longitudinal Study supplemented by college students and laboratory and hospital employees to increase the sample size of the 3rd and 4th decades. Subjects are given one of the following tests per visit: oral glucose tolerance (OGTT), intravenous glucose tolerance (IVGTT), cortisone glucose tolerance (CGTT), or intravenous tolbutamide response test (TRT). On the fifth visit the cycle is re-started. Diabetic patients are available primarily from the Baltimore City Hospitals Out-Patient Department; "chemical" diabetics and a small number of overt diabetics are available from the Longitudinal Study Group.

In a series of studies on the mechanism of the age change the servo-control "glucose-clamp" technique is used: control of arterial blood glucose is placed under the investigator's control. In some studies glucose-1-C^{14} is injected intravenously either as a single shot or as a continuous infusion following a prime. The course of the specific activity of circulating glucose is used to compute glucose production (primarily by liver), glucose uptake (equals production when steady-state glucose concentration is maintained), and glucose space. An Amberlite anion exchange resin is used to separate glucose in plasma from charged labeled metabolites of glucose and recycling error is corrected by established techniques.

Major Findings: (1) The mechanism of the deterioration in glucose tolerance with age is being further clarified by the addition of tracer technology to the glucose-clamp method. Fourteen experiments in volunteers aged 21 to 67 years were performed. The insulin kinetics in these studies are reported in a separate Project Report. Following a basal state control period, continuous intravenous insulin infusion resulted in arterial plasma insulin plateaus averaging 100 μU per ml. Hepatic glucose production in the basal state averaged 1.8 $\pm$ 0.20 (SD) mg per kg body weight per minute; there were no age differences in the non-"stressed" subject. Recycling of labeled metabolites to glucose reaches a peak of some 5% of total hepatic glucose production during the study and again there is no effect of age on the rate of recycling. Quantitation of hepatic glucose production during the insulin infusion is being performed with the collaboration of Dr. Mones Berman and depends upon the establishment and definition of an appropriate glucose and insulin mathematical model. Prelimary results indicate that hepatic glucose production decreased promptly upon insulinization so that by 20 minutes it had fallen to only 30% of the basal level and again no age differences were detected.

(2) The multiple variables which influence the glucose-insulin metabolic system (age, sex, obesity, dietary state, physical activity, diabetes, other endocrinopathies) make it essential that the informative glucose-clamp technique be adaptable to study of large numbers of subjects. Two

problems needed to be solved: (a) computational complexities in the servo-control formulae and (b) the avoidance of arterial catheterization. To solve the first problem we have collaborated with members of the Computer Systems Laboratory in order to develop a fully automated on-line system which would eventually enable us to perform several clamp studies simultaneously. The system accepts voltage peaks from the AutoAnalyzer glucose analysis, computes glucose concentrations, retains data which enables it to feed back instructions on-line for periodic readjustment of glucose infusion rates. This system is now in its final testing phase. The problem of avoiding arterial catheterization was approached by assessing a technique for "arterialization" of peripheral venous blood. An electrically heated glove, thermostatically controlled at 40°C, results in marked vasodilation of the A-V shunts in the fingers and hand. Since there is relatively little metabolically active tissue in the hand, venous effluent blood would be expected to approximate arterial blood closely. The "hot-hand" technique might then enable us to substitute venous for arterial blood. To test this idea, we have performed five glucose-clamp experiments in which basal glucose concentration was raised by 125 mg per ml. Both brachial arterial and superficial venous catheters were placed and A-V glucose differences were computed. The hyperglycemia with its resultant endogenous hyperinsulinism is a rigid test of the degree of arterialization of the venous blood, since large A-V glucose differences normally occur under these conditions. The results were gratifying in that A-V differences averaged only 2.2 mg% as opposed to average differences of 11.3 mg% when the hot-hand technique is not used. Furthermore insulin A-V differences averaged only 2.6 μU per ml. We will thus be able to use the clamp technique on the Longitudinal Subjects of the Gerontology Research Center.

Significance to Bio-Medical Research and the Program of the Institute: The ability to separate glucose production from glucose utilization by the tracer technique will add an important dimension to these studies. The question of over-production versus under-utilization of glucose as the more important factor in the glucose intolerance of aging and of diabetes may thus be resolved.

The "testing of the tests" in clinical use for the diagnosis of diabetes is a serious one. The present study fulfills most of the recommendations for future research needs made by the Committee on Statistics of the American Diabetes Association. This report dealt only with the oral glucose tolerance test. The intravenous variant of this test is gaining in popularity especially among clinical research workers interested in the relation of atherosclerosis to metabolic factors. There is however no large scale study available comparing the results to these two tests. Similarly the tolbutamide and cortisone tests remain of controversial value and their present study will offer the most extensive evidence on the relative advantages and defects of these procedures.

Proposed Course of Project: (1) The Longitudinal Study will continue with the added dimension of glucose-clamp studies in large numbers of subjects. (2) Definitive glucose-tracer modeling will be combined with the insulin modeling system in what should result in the most detailed experimental

assessment of glucose-insulin interrelations available in man. (3) Obesity and overt diabetes will be studied in order to understand their interrelations with the metabolic alterations of aging.

Honors and Awards: Dr. Andres addressed the Diabetes Center of the New York Medical College.

Dr. Andres was appointed as a member of the Baltimore City Commission on the Problems of the Aging.

Publications: Andres, R.: Effect of age in interpretation of glucose and tolbutamide tolerance tests. In Diabetes Mellitus: Diagnosis and Treatment, Vol. III, prepared under the auspices of the Committee on Professional Education of the American Diabetes Association, Inc., 1971 (In press).

Andres, R.: Aging and diabetes. Med. Clinics of North America 55: 835-846, 1971.

Serial No. HD-CP7(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The kinetics of insulin metabolism in man: relations to glucose metabolism, to age, and to obesity.

Previous Serial Number: New Project

Principal Investigators: Robert S. Sherwin
Jordan D. Tobin
Reubin Andres

Other Investigators: John Liljenquist
Paul Insel

Cooperating Units: Mones Berman
Mathematical Research Branch, NIAMD

Man Years:
Total: 3.05
Professional: 2.00
Other: 1.17

Project Description:

Objectives: These studies are directed at defining insulin kinetics in man in order to understand the changes in glucose and insulin responses in man as he ages, as he develops diabetes, and in relation to obesity. The servo-control (glucose-clamp) studies done in this laboratory have demonstrated lower arterial plasma insulin levels in response to controlled hyperglycemia with increasing age. The interpretation of this finding requires consideration of the insulin release, distribution, and clearance mechanisms. Current studies are aimed at defining these variables.

Methods Employed: The glucose-clamp technique was used in order to maintain each subject at his own basal arterial blood glucose concentration despite experimental perturbations of the arterial plasma insulin level. The clamp technique consists of a controlled variable-rate IV infusion of a glucose solution with rapid measurement of blood glucose concentration by an automated method. A negative feed-back formula is applied at five minute intervals (as blood glucose concentrations become available) in order to servo-correct the glucose infusion rate; thus the blood glucose

concentration is maintained by euglycemic levels.

Intravenous infusions of insulin were given by two techniques (1) single shot intravenous insulin injection, 0.025 units crystalline porcine insulin per kg body weight and (2) prime plus continuous infusion of insulin. The prime was given over a 10 minute period such that the total insulin infused in that time was twice the continuous rate infused from 10 to 80 minutes. The infusion during the 10 minute prime was at a logarithmically falling rate which essentially approached the continuous infusion rate asymptotically. The goal of this technique was to create a nearly instantaneous square wave of insulinization at a new steady-state level. Studies were carried out at continuous infusion rates of 1 and at 2 mU per kg body weight per minute.

Major Findings: (1) The single-shot insulin studies (6 young adults) were first analyzed by the conventional method of assuming that the falling insulin concentration curve described a single slope. Insulin half-times averaged 10 minutes (if absolute insulin concentration is used) or 6 minutes (if basal insulin level is substrated from each absolute level), values which agree with those published by others.

(2) Insulin plateau levels achieved by the continuous infusion studies averaged 99 and 198 μU per ml for the 1 and 2 mU per kg per min. experiments respectively and did not differ with age.

(3) The metabolic clearance rate of insulin (MCR) is defined by the insulin infusion rate divided by the plateau insulin concentration achieved.

In the table below, body surface area is used as the reference base in order to compare non-obese with obese subjects. Means and standard deviations are given:

Insulin Infusion Rate (mU per kg per min)	Young Adults (Normal wt)	Old Adults (Normal wt)	Young Adults (Obese)
1	481±68 (14)	423±65 (7)	--
2	420±70 (7)	456±21 (4)	391±73 (4)

These results show that there is no significant effect of age or of obesity on insulin clearance. They also demonstrate that clearance is uninfluenced by insulin dosage at these levels.

Previous studies in this laboratory have demonstrated that old subjects have lower insulin levels than do young subjects when presented with equivalent hyperglycemic stimuli.

This difference must be due to decreased beta cell sensitivity with age; differences in insulin clearance rates with age are not responsible for age differences in plasma insulin.

(4) On the basis of the clearance rates computed above, the endogenous basal "post-hepatic" or systemic insulin delivery rates can be computed:

Delivery rate (basal)=Insulin clearance rates x Insulin conc. (basal)

In young and old subjects delivery rates were nearly identical, averaging 128 and 125 mU per kg body weight per min.

Significance to Bio-Medical Research and the Program of the Institute: Half of the older population of the U.S. have abnormal glucose tolerance and are therefore considered by some to be diabetic; others consider this decline in tolerance to be normal or physiologic. It is therefore of great public health importance to gain a thorough understanding of the physiology of aging and the pathophysiology of diabetes in order to resolve this dispute. We have therefore exerted great effort to design experiments in man which yield more detailed information on the mechanisms involved in glucose and insulin metabolism than have previously been possible.

Proposed Course of Project: We have recently begun a collaboration with Dr. Mones Berman, Mathematical Research Brance,NIAMD for multicompartmental analysis of these studies, using the versatile SAAM 25 program of Berman and Weiss to compute compartment dimensions and intercompartmental transfer rates. The time course of insulin build-up and decay in the several compartments will be related to the time course of insulin effect as measured by glucose utilization and endogenous glucose production rates.

Honors and Awards: None

Publications: None

Serial No. HD-CP8(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Prediabetes: Sensitivity of beta cells to hyperglycemia and sensitivity of tissues to insulin

Previous Serial Number: New Project

Principal Investigators: Jordan Tobin

Other Investigators: Robert S. Sherwin
Paul A. Insel

Cooperating Units: J. Stuart Soeldner
E.P. Joslin Research Labs
Boston, Massachusetts

R. Camerini-Davalos
New York Diabetes Center
New York Medical College
New York, New York

Baltimore City Hospitals

Man Years:

Total:	0.40
Professional:	0.20
Other:	0.20

Project Description:

Objectives: Prediabetics are defined as subjects with a strong family history of diabetes (offspring of 2 diabetic parents, or identical twin of a diabetic), who therefore are likely to be genetic diabetics, but who, up to the time of study, have normal glucose tolerance. They have been shown to have a decreased early insulin response to a hyperglycemic challenge. Since their glucose tolerance is normal, they have been thought to have an increased sensitivity to the insulin they do secrete.

Since the glucose-clamp technique quantitates both beta cell sensitivity to hyperglycemia and sensitivity of tissues to the endogenous insulin, this technique was used to study these metabolic parameters in prediabetes.

Methods Employed: Seven normal males, aged 23 ± 3.7 (SD) years and seven prediabetic males (4 offspring of 2 diabetic parents and 3 identical twin of diabetics) with a mean age of 29 ± 3.1 years were studied. The prediabetics all had normal oral, cortisone-primed oral, and intravenous glucose tolerance tests. There were no significant differences between the two groups in age, height, weight, obesity index, fasting blood glucose or fasting plasma insulin concentrations.

Using the clamp technique the fasting blood glucose level was raised by 125 mg% and maintained at that level for 2 hours.

Major Findings: Glucose metabolized (M) was lower in the prediabetics as a group but this was statistically significant only from 60 to 80 minutes in the study.

The plasma insulin values (I) were qualitatively similar in both groups. In response to the square wave of hyperglycemia there was a biphasic insulin pattern with a peak response from 4-6 minutes, a fall to a nadir from 10-15 minutes, and a progressive increase during the remainder of the test. Peak values in the prediabetics were significantly lower than in the normals, the 4 minute value being 50% of that in the normals ($P<.01$) and the 6 minute value 60% ($P<.05$). Thereafter,though the mean values remained lower in the prediabetics, this difference was no longer statistically significant.

The sensitivity of the insulin-responsive tissues of the body can be estimated from the relationship during the course of the study, between the glucose utilized, M, and the arterial insulin concentration curve, I, to which the tissues are exposed. In essence I and M are the measures of a dose-response relationship. Thus the M/I ratio could be computed as a measure of sensitivity to insulin, the implied assumption being that the dose-response curve is simple and linear. Computed this way, sensitivity to insulin is not significantly different between the two groups.

Significance to Bio-Medical Research and the Program of the Institute: Using the glucose clamp technique to place both prediabetics and normals under the same steady-state hyperglycemic stimulus we have demonstrated that prediabetics as a group tend to have lower glucose metabolism (M), and lower insulin levels (I) throughout the test but of statistical significance only at the early times. These findings agree with previous studies on prediabetics which have demonstrated an early insulin deficit in response to I.V. glucose.

The sensitivity of the body to endogenous insulin, M/I, is not different between the 2 groups however since both M and I are slightly lower in the prediabetics.

Diabetes has been extensively studied in an attempt to define its pathophysiology and especially with respect to the relation between the metabolic abnormalities observed and the known complications of the disease. Genetic prediabetics have been studied in an effort to elucidate the earliest metabolic defect in the natural history of the disease. This is also

important since patients do present with the complications of the disease before any metabolic abnormality is noted.

A basic goal of this Section is to gain an understanding of the mechanisms of the decline in glucose tolerance with age, and specifically to determine whether this decline is an expression of the development of diabetes or whether it represents a physiological change of aging. Characterization of the evolution of the early diabetic state (prediabetes) and its relation to the changes that occur with aging should help in this endeavor.

Proposed Course of Project: The M/I ratio as a measure of sensitivity to insulin is a preliminary computation and more sophisticated analysis of the data is necessary in order to derive from the arterial plasma insulin values its rate of distribution into its several spaces. By use of models derived from the SAAM 25 program various parameters of insulin distribution will be related to time course of the glucose metabolized; a more reliable estimate of sensitivity to insulin should result.

Honors and Awards: Dr. Tobin was an invited speaker at a symposium sponsored by the Philadelphia Geriatric Center and the Philadelphia County Medical Society on "Hyperglycemia and Nutrition in the Aged" on November 4, 1970.

Publications: None

Serial No. HD-AG21(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The renin-angiotensin system. I. Chemical-radioisotope derivative assay of plasma renin and angiotensin. II. Labeled substrate assay for plasma renin.

Previous Serial Number: HD-AG 21

Principal Investigators: Nicholas M. Bath
J. Howard Lutz
Robert I. Gregerman

Other Investigators: None

Cooperating Units: Division of Endocrinology and Metabolism
University of Michigan Medical Center

Baltimore City Hospitals

Man Years:

Total:	4.35
Professional:	4.35
Other:	0.00

Project Description:

Objectives: Until recently regulation of the secretion of aldosterone, the adrenal's principal mineralocorticoid, has been thought to be chiefly controlled under physiologic conditions by the release of renin, a proteolytic enzyme, from the kidney into the circulation. The enzyme cleaves the decapeptide, angiotensin I, from a plasma α_2 globulin. The decapeptide is then "converted" in the lung to the biologically active octapeptide, angiotensin II. Two recent developments underline the need for specific, accurate methods for the measurement of renin. First, on the basis of bioassay results, the role of renin as the regulator of aldosterone has very recently been seriously challenged. Second, limited studies strongly suggest that the determination of renal vein renin by bilateral catheterization is by far the most definite diagnostic method for the detection of hypertension due to unilateral reduction in blood flow. A ratio of 1.5 between kidneys on the two sides appears to be diagnostic. Both developments provide the impetus for further methodologic refinement of highly specific and accurate techniques for measurement of renin in plasma. The

role of renin as a regulator of aldosterone will have to be restudied using methods more specific than those of bioassay, while a high degree of accuracy will be required in clinical work with the diagnosis of renovascular hypertension. Our laboratory has been concerned with several methodologic approaches.

Methods Employed: Our first effort has been the development of a double isotope derivative method for angiotensin I, the peptide produced by renin, and application of this technique to renin measurement.

An alternative approach has been to develop an entirely new technique for the measurement of the enzyme. For this a labeled substrate has been synthesized and evaluated as substrate for renin and pseudorenin. Suitability of the substrate for the assay of renin in plasma has been studied.

Major Findings: (1) The double isotope assay has been evaluated for renin measurement in human plasma. Renin is measureable in the plasma of all normal subjects studied to date. When individuals are given a normal sodium intake (approximately 100 mEq per day) additional sodium (total 200 mEq per day) does not appear to lower the renin level of recumbent subjects, although the normal response to upright posture, an increase of the enzyme, may be somewhat reduced. These results are in agreement with some bioassay results and at variance with others which have claimed complete suppression of renin with a high salt intake. When a diuretic (furosemide) is given to normal subjects, plasma renin rises sharply. These results are in agreement with those from bioassay. The ranges of response have not yet been completely determined, but are under study. Data on reproducibility and accuracy continue to be gathered.

(2) The synthetic labeled polymeric substrate, prepared for the first time in our laboratory, is N-acetyl-poly-L-glutamyl (M.W. 50,000)-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser labeled with ^{125}I on the two tyrosines. Our findings with this substrate are the following: [a] the substrate is cleaved by human renin prepared by the method of Haas; by human renin, free of pseudorenin, prepared by the method of Waldhausl, et al.; and by beef spleen pseudorenin, prepared by the method of Lentz, Skeggs, et al. The product is the expected tetrapeptide Leu-Val-Tyr-(I^{125})-Ser which has been identified by multiple thin layer chromatographic systems and electrophoresis. [b] Equal activities of Haas and Waldhausl renin, measured by their activities on hog globulin renin substrate in the double isotope method, have different activities against the polymeric substrate. Haas renin shows about 5 times more activity in the latter assay. The difference is probably due to the presence of pseudorenin in the preparation or, less likely, to alteration of the enzyme in the Waldhausl isolation. [c] The pH optimum for the various enzyme preparations have been studied and are somewhat different, but are in the range of pH 4.5 to 5.5. As expected, the pH optimum with pseudorenin is lower. [d] Kinetic measurements have shown that the K_M with this substrate (concentration of substrate for half-maximal velocity) is 0.2×10^{-6} M. This substrate has a higher affinity (lower K_M) for the enzyme than does the natural protein substrate of plasma (K_M 1.2×10^{-6} M) and the uncoupled synthetic tetradecapeptide (K_M 2×10^{-6} M). The maximum

reaction velocities are similar for the synthetic substrate and the natural protein. [e] The activity of the enzyme with polymeric substrate in buffer is 40 times faster than in plasma, indicating inhibition of renin by the proteins in plasma. This finding needs further investigation, but is not entirely unexpected. Plasma contains a number of proteinase inhibitors. To date none has been identified for renin or pseudorenin, but further work along these lines is of obvious interest and possible importance. [f] The pH optimum for endogenous plasma enzyme activity against the synthetic substrate is 4.5. At this pH, apparent renin activity is 2 to 10 times that determined by double isotope assay for angiotensin I. The pH 4.5 activity is seen in the presence of EDTA and DFP. Most of the product of enzyme action under these conditions is the expected tetrapeptide. These observations suggest that enzyme activity at pH 4.5 is due to pseudorenin rather than to renin. Although these data indicate that the rate of attack of the synthetic polymeric labeled substrate is several orders of magnitude slower than that of free tetradecapeptide, the polymer is not immune from attack by plasma pseudorenin and is therefore not a suitable substrate under these conditions.

At pH 5.5 the activity of pseudorenin in plasma is very much reduced, but it remains to be seen whether at higher pH, the observed renin activity approaches the levels measured by conventional assays. At these higher pH values, the sensitivity of the polymeric substrate assay may become a limiting factor, but work along this line is in progress.

Significance to Bio-Medical Research and the Program of the Institute: Hypertension is a major, age-related disease complex which affects millions of persons and inflicts enormous morbidity and mortality. The development of these techniques will enhance both the basic understanding of disease mechanisms and facilitate the practical diagnostic evaluation of patients.

Proposed Course of Project: Data will continue to be gathered on accuracy and reproducibility of the double isotope method. Technical simplification will be evaluated. An immunoassay for angiotensin I, currently in increasing use elsewhere, is being evaluated in our laboratory. Results with immunoassay will be compared directly with our double isotope technique. Physiologic measurements will continue to be made. An effort will be made to explore in some detail the interrelationships between plasma renin (by our double isotope method) and the secretion of aldosterone. This will represent a collaborative effort with investigators at the Johns Hopkins University.

The labeled substrate assay will proceed in two directions. First, an effort will be made to remove or selectively inhibit the pseudorenin activity which is probably responsible for our results with plasma to date. Differential assay of renin and pseudorenin will be attempted by careful manipulation of pH. Human pseudorenin will be prepared by DEAE chromatography of crude human renin, antibodies formed to the pseudorenin, and an attempt made to inhibit selectively the pseudorenin of plasma in order to permit the assay of renin with the present labeled polymeric substrate. Regardless of our future efforts with plasma assays, the present polymeric

labeled substrate assay is the simplest, most rapid technique for renin measurement devised to date. It should be extremely useful in its present form for study of enzyme inhibitors, purification of enzyme, etc.

Another approach will be used simultaenously. An entirely new labeled substrate will be synthesized in the hope of imparting greater specificity to enzymatic cleavage. A reverse coupling will be done with poly-L-lysine in which the C-terminal and of the tridecapeptide becomes bound to the polymer and the N-terminal arginine is free. Thus, enzymatic attack will produce a labeled nonapeptide (des-aspartyl-angiotensin I-^{125}I). Preparation of carrier and indicator nonapeptide will be by enzymatic cleavage of the available tridecapeptide to 9 and 4 amino acid fragments,or by solid phase synthesis, or both.

Honors and Awards: Dr. Gregerman presented invited lectures on "Double isotope and labeled polymeric substrate assays for renin" at the Department of Medicine, University of Oklahoma Medical Center, November, 1970, and the U.S. Army Research Institute of Infectious Diseases, Fort Detrick, Maryland, October 1970.

Publications: Waldhausl, W.K., Lucas, C.P., Conn, J.W., Lutz, J.H., and Cohen, E.L.: Studies on the partial isolation of human renin. Biochim. Biophys. Acta 221: 536, 1970.

Gregerman, R.I. and Kowatch, M.A.: Double isotope assay for angiotensin I: Use for renin measurement. J. Clin. Endocrinol. and Metab. 32: 110-114, 1971.

Serial No. HD-AG22(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Relationship of age to thyroid function and thyroid hormone metabolism

Previous Serial Number: HD-AG 22 (c)

Principal Investigators: Robert I. Gregerman
Paul J. Davis (Resigned 9/70)
Stephen W. Spaulding (Resigned 6/70)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	0.60
Professional:	0.60
Other:	0.00

Project Description:

Objectives: Previous studies from this laboratory have shown an age dependent decrease of thyroid function secondary to decreased thyroid hormone (T4) disposal. The mechanism of this change is unknown. Our work in this area represents efforts to provide answers to these questions:

(1) What are the factors which control the rate of thyroxine metabolism and hence the age-related alteration of thyroxine disposal?
(2) Does the transport of T4 affect metabolism?
(3) What are the factors which control the level of "free" (non-protein bound hormone) in plasma?

Studies in the past year have been related to the second and third questions. We have completed the previously reported study of protein binding of T4 in an animal model (the rat) and undertaken certain physico-chemical studies of T4 binding to human plasma proteins.

Methods Employed: Polyacrylamide gel-slab electrophoresis studies of T4 binding have been performed as described in a previous report. Equilibrium dialysis has been used to study the dilution and ionic effects described below.

Major Findings: Binding of T4 to plasma proteins of the rat has been de-

fined. The previous findings that T4 binding in the rat and man are quantitatively different but qualitatively very similar are confirmed. Thyroxine-binding-prealbumin (TBPA) is the major carrier of T4 in the rat, as opposed to thyroxine-binding globulin (TBG) in man. Inhibitor studies confirm the qualitative similarities between the protein patterns of the two species and indicate that the rat may be a suitable model for a variety of studies including those factors controlling free hormone levels in man.

It has been known that for some years, in equilibrium dialysis measurements employing labeled thyroxine, dilution of serum results in a decrease in the percent free thyroxine (PFT) ranging between 25 and 40 percent. This observation is contrary to the prediction from the law of mass action that the free thyroxine should actually increase slightly. Removal of weakly bound contaminants from the labeled thyroxine does not eliminate the dilution effect, although previous work suggested that this was the case. Rather, the dilution phenomenon appears to be largely explained by ionic effects. Both chloride and phosphate ions are shown to inhibit thyroxine binding to serum proteins and raise the free thyroxine concentration. The chloride effect is proportional to increasing concentration over a wide range, but the phosphate effect does not increase beyond 0.05 M. Thus, when serum is diluted with phosphate buffer, the PFT decreases in a manner predictable from the final equilibrium concentrations of chloride and phosphate in each sample. The PFT is very sensitive to changes in pH. The magnitude of the pH effect is inversely related to the concentration of the buffer used.

Free thyroxine is reported to be elevated in renal insufficiency. The present work demonstrates that a number of compounds known to be present in elevated concentrations in uremia also elevate the PFT. These compounds include indole, several of its naturally occuring congeners, and a variety of phenols. The effects of inorganic ions may explain much of the dilution effect in normal serum, but the findings of other naturally occuring small molecular weight compounds that affect the PFT is consistent with the possibility that compounds with similar effects are responsible for the elevated free thyroxine of certain disease states.

Significance to Bio-Medical Research and the Program of the Institute: This work serves to develop our understanding of basic mechanisms of thyroid physiology and biochemistry. The age-related changes of thyroid hormone economy which we have previously demonstrated will be further understood only with development of new information in this area.

Proposed Course of Project: Further efforts will be made to define the nature of the suspected plasma inhibitors of T4 binding to human T4 binding proteins. A manuscript describing the dilution effects and binding inhibition by specific ions has been prepared and submitted for publication.

Honors and Awards: None

Publications: Davis, P.J., Spaulding, S.W., and Gregerman, R.I.: The three thyroxine-binding proteins in rat serum: binding capacities and effect of binding inhibitors. Endocrinology 87: 978-986, 1970.

Spaulding, S.W. and Davis, P.J.: Thyroxine binding to soluble proteins in rat liver and its sex dependence. Biochem. Biophys. Acta 229: 279-283, 1971.

Serial No. HD-CP9(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section and Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chlorpropamide hyponatremia: an inappropriate antidiuretic hormone (ADH) syndrome

Previous Serial Number: HD-CP 9 (c)

Principal Investigators: Robert I. Gregerman
Peter N. Weissman (Resigned 6/70)
Louis Shenkman (Resigned 6/69)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:
Total: 0.05
Professional: 0.05
Other: 0.00

Project Description:

Objectives: Chlorpropamide is used daily in the United States by an estimated 400,000 persons with diabetes mellitus. Although the drug's blood sugar lowering action has been known for over a decade, its action as an antidiuretic compound has been known for only a few years. Because of this latter effect we suspected that patients receiving chlorpropamide for the treatment of diabetes mellitus might be prone to the development of excessive water retention. Acting on this suspicion we sought and discovered 5 patients with clinical illness resulting from this previously unrecognized complication of chlorpropamide therapy.

Methods Employed: These have been previously described (Annual Report, 1969-1970).

Major Findings: In these 5 patients symptomatic hyponatremia developed during chlorpropamide therapy for diabetes mellitus. Chlorpropamide administration was associated with hyponatremia and serum hypoosmolality, continued sodium excretion despite hyponatremia and an impaired ability to dilute urine maximally and to excrete a water load. Clinical and

chemical abnormalities were corrected by withdrawal of chlorpropamide and reappeared when the drug was readministered. The phenomenon represents the first example of a drug-induced reversible form of the syndrome of inappropriate activity of antidiuretic hormone due to enhancement of action by the drug. The reason for the development of hyponatremia in only a small percentage of persons receiving the drug is not clear. The syndrome was seen in 4 per cent of patients receiving chlorpropamide in a clinic population. This may be related to individual variations in ability to suppress endogenous antidiuretic hormone in response to hypoosmolality. It has been estimated that more than 10,000 persons may currently be experiencing morbidity due to this drug.

Significance to Bio-Medical Research and the Program of the Institute: This work is related to the age-dependent alterations of carbohydrate metabolism and diabetes mellitus. It has uncovered a new complication of diabetes therapy with oral drugs, and may have special relevance to therapy of the aging diabetic subject.

Proposed Course of Project: This study has now been terminated. The results have been published. No further work on this project is contemplated for the coming year.

Honors and Awards: None

Publications: Weissman, P.N., Shenkman, L., and Gregerman, R.I.: Chlorpropamide hyponatremia: Drug induced antidiuretic hormone activity. New Eng. J. Med. 284: 65-71, 1971.

Serial No. HD-CP11(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Lymphocyte responses to specific thyroidal antigens and the pathogenesis of hyperthyroidism (Graves' Disease)

Previous Serial No.: New Project

Principal Investigators: Barry S. Handwerger (85% time)
Robert I. Gregerman (20% time)

Other Investigators: Paul J. Davis*
David Brandes*

Cooperating Units: *Baltimore City Hospitals

Man Years:
Total: 0.80
Professional: 0.80
Other: 0.00

Project Description:

Objectives: The plasma of most individuals with hyperthyroidism (Graves' Disease) contains a protein with thyroid stimulating activity (long acting thyroid stimulator; LATS) similar in action to thyrotropin but having a slower onset of action and more prolonged effect. This protein has the chemical characteristics of a 7-S gamma globulin. It has been postulated that LATS is an antibody produced in response to stimulation by an antigen from the thyroid gland. By some process, presumably a destructive one, thyroidal protein is thought to reach immunologically competent cells and to produce an immune response, the result of which is the formation of LATS.

Recently the lymphocytes of patients with hyperthyroidism have been shown to produce LATS during culture in vitro following activation with the non-specific lymphocyte transforming substance, phytohemaglutinin (PHA). Moreover, lymphocytes from immunologically sensitized individuals can be stimulated by specific antigens. For example, lymphocytes from a tuberculin sensitive individual with a positive delayed sensitivity skin test to purified protein derivative (PPD) of tubercle bacilli will be stimulated by PPD during in vitro culture.

Methods Employed: Lymphocytes from normal and hyperthyroid subjects are

grown in vitro in short term culture by standard techniques. Immunologic stimulation is assessed with non-specific (PHA) and specific antigens. The lymphocyte responses are measured in two ways. First, the incorporation of tritium labeled thymidine into lymphocyte DNA gives an easily measured index of response (blastogenesis) within 7 days. When evidence of such a response is observed, specific antibody can be tested for by appropriate immunologic methods or by bioassay.

Human thyroid tissue has been obtained from surgical specimens and from autopsy material and fractionated into soluble, mitochondrial, microsomal, and nuclear fractions. Each will be tested for blastogenic effect. Further fractionations will be made as indicated.

Major Findings: The lymphocyte culture technique has been standardized to an acceptably reproducible level as judged by the thymidine incorporation technique. Both PHA and PPD stimulation have proven predictable and dose dependent. Human thyroid fractions have been prepared.

Significance to Bio-Medical Research and the Program of the Institute: Hyperthyroidism appears to be an immunologic disease but the mechanism of its pathogenesis is still unclear. Demonstration of lymphocyte transformation and specific antibody (LATS) formation following stimulation by thyroid antigens will constitute definitive proof that LATS production in Graves' Disease is due to auto-sensitization by specific thyroid antigens. Further understanding of decreased antibody production by human lymphocytes and the appearance of tissue specific antibodies during aging will be advanced by such insights into the common auto-immune diseases.

Proposed Course of Project: Stimulation of lymphocytes with specific thyroid fractions will soon be attempted. If blastogenesis can be demonstrated with these techniques, further protein fractionation will be performed in an attempt to define chemically the antigens responsible, while specificity of the response will be measured with a bioassay technique for LATS. An attempt will be made to assay LATS by stimulation of thyroidal adenyl cyclase.

Honors and Awards: None

Publications: None

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1971
Office of the Associate Director for Extramural Programs

Research and training in Perinatal Biology and Infant Mortality, Growth and Development, Mental Retardation, and Adult Development and Aging was continued during fiscal year 1971. As of July 1, 1970, all research and training in developmental biology which had been included in the Center for Population Research, NICHD, was transferred to the Extramural Programs. All of the training and most of the research grants were placed in the Perinatal Biology and Infant Mortality Branch.

The Institute's Research and Training Committees have adapted very well to the additional burden placed upon them by the responsibility of providing initial review for program projects in addition to training grants, research career development awards and fellowships. Fiscal year 1970 was the first full year of operation under this new policy and I am pleased to report that the results were excellent. The reviewers demonstrated an exemplary conscientiousness with regard to maintaining high standards and showed a willingness to work long hours in order to be certain that reviews were thorough.

During the year it seemed to many of us that closer liaison between our Council and the Research and Training Committees would be desirable. Accordingly, each Chairman was invited to attend meetings of the Council, beginning with the March 1971 meeting. All Chairmen were able to attend and found the sessions both interesting and useful. During the discussion of applications they were able to respond to questions from Council regarding Committee actions during the initial review. At the subsequent meetings of the Research and Training Committees, each Chairman reported his general impression of the Council meeting. It was clear that Committee Members appreciated learning more about how the Council functions. Thus, our original expectations were fully confirmed, namely, that both Council and the Research and Training Committees would benefit by having the Chairmen meet with Council. The Institute plans to continue inviting them.

From its inception, the Institute has encouraged its Extramural staff to engage in the active programming of applications. We are convinced that this is an important reason for the steadily increasing interest in the programs of the Institute as evidenced by the fact that at almost every Council meeting the number of applications exceeds the number at the previous Council meeting. This occurs in spite of the fact that larger and larger numbers of approved applications cannot be funded. (More than 500 in fiscal year 1971, with an estimated dollar value of over $22 million.) The possibility that an approved application might not be funded is always carefully explained to a potential applicant. It is also made clear, however, that the Institute intends to maintain its policy of giving encouragement to new ideas and new applicants. Even so, the process of active programming is approached with some trepidation

by staff because of the many potential pitfalls which lie in the path of any application. Nevertheless, we feel that because of the great importance of the fields for which we are responsible, the activity must continue even in times of financial difficulty.

In addition to continuing the active programming of grants, the Extramural scientific staff intensified its efforts to encourage certain types of research via the contract mechanism. Plans were laid during fiscal year 1970 in order that the multiple review process could be accomplished well before the spring of 1971. These plans were thwarted by the imposition during fiscal year 1971 of a new requirement that requests for proposals must be sent out to a number of prospective contractors even when the research is to be done in a university (prior to this change, sole source could be recommended for university contract research.) Not only did this new regulation greatly lengthen the review process, but in addition it greatly increased the amount of work both on the part of Extramural and Program Services staff. In terms of timing, the real difficulty was caused by the fact that we were not informed of the new requirement until fiscal year 1971 was well underway. I am pleased to report, however, that as a consequence of sending out requests for proposals the quality of contracts eventually approved was increased.

The fact that support of research training continued to become more and more difficult during fiscal year 1971 was especially hard on the NICHD because many of the areas we are responsible for are relatively new on the academic scene, for example, perinatology and mental retardation. The number of investigators needed continues to increase in spite of the fact that funds for training grants, research career development awards and fellowships do not increase.

All of the Extramural Programs continued to hold conferences during the year, from many of which publications will eventually appear. We continue to regard both conferences and the ensuing publications as extremely important aspects of the work of the Institute. The conferences range from workshops involving a very small number of experts to large multi-disciplinary meetings, but in all cases they contribute to the support of the many missions of the NICHD.

The Extramural Programs regretted the loss of three staff members during the year. Dr. William Moore left the Growth and Development Program to assume the position of medical director of Ross Laboratories; Dr. James Teegarden left the Growth and Development Program to become the Executive Secretary of the Alcoholism Panel in NIMH; and Dr. Suzanne Stimler left the Aging Program for a position in the Division of Research Resources.

The Institute was fortunate in being able to recruit the following new individuals during the year: Dr. Sigmund Dragastin, a sociologist, joined the Growth and Development Program; Dr. Mark Fitzpatrick, an obstetrician, joined the Perinatal Biology and Infant Mortality Program; and, Dr. John McKigney, a nutritionist, joined the Growth and Development Program. Additional recruiting efforts are continuing as this is written.

Highlights of the activities and accomplishments of the Extramural Branches are detailed in the reports which follow.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Perinatal Biology and Infant Mortality Branch

The objective of the Perinatal Biology and Infant Mortality Branch is the promotion of a coordinated program of research and training which will result in the contribution of new knowledge toward the achievement of maximum health and well being for every pregnant woman and her progeny. Program objectives also strive to achieve a marked reduction in this country's infant mortality rate, to ameliorate infant morbidity, and to narrow the gap between the identification of new knowledge and its incorporation into the delivery of health care.

Within the scope of the program, emphasis is placed upon increased understanding of both normal and abnormal processes involved in pregnancy maintenance, management, and termination. Of equal importance is the achievement of a better understanding of mechanisms and processes involved in embryonic development and fetal growth, and of problems with which the newly born infant must cope in his adaptation to extrauterine life and subsequent survival and well being. Particularly relevant are those morbidity- and mortality-related health problems to which the infant may be subjected during the first year of life.

The Branch supports research and training concerned with various aspects of the physiology of pregnancy; disorders of pregnancy; placental function; immunologic phenomena; developmental biology and physiology; and the biochemical processes of embryonic and fetal growth; the process of labor and delivery, including biophysical and biochemical diagnostic procedures; and genetic, pharmacologic, toxic, and infectious processes and influences which can affect the mother and fetus. Primary attention in the area of infant survival and well being emphasizes respiratory function, metabolic processes, immunology and blood dyscrasias, infectious diseases, congenital malformations, and sudden unexpected death.

Program objectives recognize the interrelationships of specific health and developmental problems emcompassed in the perinatal and infant periods of life and the effects of events occuring during these periods upon subsequent development and well being. Thus, emphasis is placed upon the interrelationships of specific problems which span program and problem areas. For example, drug ingestion by an otherwise healthy gravida can influence fetal survival and development. Toxemia of pregnancy can result in a high risk neonate. Early termination of pregnancy increases the risk of the newborn developing respiratory distress syndrome. Events occuring during the period of hospitalization following birth can markedly influence the subsequent behavior and development of the baby.

The Perinatal Biology and Infant Mortality Branch is responsible for the supervision of approximately 25 percent of the Institute's extramural holdings, totaling about 299 grants and representing over 12.4 million dollars. Approximately one quarter of the Program's grant holdings are concerned with training support. Funds involved for training approximate 3.2 million and 25 percent of PBIM funds. These funds support 29 research

training grants in reproductive physiology, developmental biology, fetal physiology, teratology, neonatal biology, human embryology, maternal-fetal relationships, pathophysiology of prenatal and neonatal life, perinatal medicine and obstetrics. A majority of these training programs are conducted in Departments of Obstetrics and Pediatrics. Thirty research career development awards were made to young investigators with exceptional potential for productive research in perinatal biology. Fellowship support was awarded to twelve others. The increase in program size as related to grant holdings and funds reflects the transfer of developmental biology activities and grant holdings to the PBIM Branch from the Extramural Program of the Center for Population Research. This transfer took place on July 1, 1970.

The PBIM Program is organized into four sub-programs: Pregnancy and Maternal Health, Developmental Biology, Fetal Health and Development, and Infant Survival and Well Being. Research grants, training grants, fellowships, and research career development awards are assigned to one of these sections by the NICHD Program Statistics Branch according to assignment criteria developed this year. Each sub-program is under the direct supervision of a Health Scientist Administrator with scientific and professional preparation pertinent to the sub-program's content emphasis. Currently no one professional person is responsible for the Infant Survival and Well Being sub-program; related responsibilities are shared by all staff.

Areas of concern and interest for each of the four sub-programs are highlighted in the following paragraphs; selected scientific accomplishments are also included.

Pregnancy and Maternal Health

Within the area of Pregnancy and Maternal Health, the PBIM Branch supports research and training concerned with various aspects of the maternal continuum including the antepartum, intrapartum, and puerperium periods. Specific diseases and disorders such as toxemia, isoimmunization, anemia and blood dyscrasias, other hemorragic phenomena associated with pregnancy, malnutrition, pernicious viral and bacterial agents are some of the conditions under investigation with grant support provided by PBIM.

Other areas of research include analysis of various physiologic changes and events (pregnancy, parturition and lactation) wrought by pregnancy. To more fully understand the underlying mechanisms pretaining to these normal maternal events, more information is needed about the contributions made by the endocrine glands, respiratory tract, cardiovascular system and genitourinary tract both of the mother and fetus. Any efforts to reduce reproductive wastage must be heralded by the application of techniques derived from acquired knowledge as well as from research concerning functions of the placenta, psycho-social dynamics of pregnancy, factors involved in the maintenance of pregnancy, and the initiation of labor as well as appreciation of intra- and extrauterine environmental hazards be they drugs, chemicals, anesthetics, or common pollutants.

Some of the scientific accomplishments achieved through grant support from this section are summarized in the following paragraphs.

Termination of pregnancy as a medical modality has become widely accepted in the last year. It is anticipated that the number of women undergoing this treatment in the near future will continue to increase at a rapid rate. It has been empirically suggested that in order to prevent isoimmunization to the Rh factor, an injection of Rh_o (D) immune globulin (human) be provided the unsensitized Rh (D) negative mother following the surgical or suction currettage of a conceptus fathered by an Rh positive male. However, one scientist, Dr. Flossie Cohen at Children's Research Center of Michigan failed to find fetal cells in the mother following this surgical procedure. Furthermore, no antibody formation was noted in these women whether they were treated by passive immunization or not. The longest gestation which occurred in this group was six months.

Of equal significance are her recent findings that some transplacental hemorrhage occurs following amniocentesis. She has found fetal cells in the maternal circulation following this procedure in 20 to 30 percent of the cases studied. Furthermore, complications following amniocentesis include an increase in maternal antibody 30 to 40 percent of the time. This phenomenon, not yet understood, has been noted to occur in pregnancies as early as 18 weeks. Dr. Cohen, therefore, suggests more judicious use of amniocentesis in the monitoring of intrauterine erythroblastotic disease since there is evidence that pregnancy outcome may be adversely affected by this iatrogenic manipulation.

In a corollary study, this investigator has studied infants transfused with immunologically competent lymphocytic cells of the opposite sex; an exceedingly slow rejection phenomenon apparently exists. She proposes, therefore, to study the developmental pattern of these infants and to assay their levels of immunoglobulin production as compared to non-transfused controls. Also proposed is a study to analyze the response of maternal lymphocytes to paternal red blood cells in an attempt to discover methods of predicting the mother's likelihood of developing significant isoimmunization to blood groupings and Rh factors.

Recent years have witnessed an increasing interest in studies concerned with bacteriuria and pregnancy. Dr. George Mitchell at St. Margaret's Hospital, Boston, discovered that the phagocytic and bactericidal activity of leukocytes isolated from pregnant women is greater than that observed in the leukocytes of non-pregnant women or in those whose pregnancy was complicated by bacteriuria. Understanding this phenomenon may help in understanding persistence of bacteria during pregnancy in spite of vigorous antimicrobial therapy. Laboratory evidence called attention to the observation that hydrogen peroxide (H_2O_2) and myeloperoxidase are two essential components of the bactericidal system in the phagocyte. During the past year, this investigator studied effects of different hormones on the intracellular events in the phagocyte. Equivocal results of increased H_2O_2 production were noted in the cells of normally pregnant women treated with estrogen, progesterone, or chorionic-gondadotrophin. However, an increase in phagocytosis, including uptake and killing effect, was noted in cells taken from pregnant women with concomitant bacteriuria when treated with an estrogen-progesterone mix. This investigator is now using

this phagocytosis model in a study concerned with estrogens and progesterones as anti-ovulatory agents and for management of post-menopausal symptoms.

Heretofore, a number of hormonal and enzymatic entities have been used to assess fetal-placental viability including such presumed measures of placental function as human chorionic gondotrophic HCG, human placental lactogen HPL, plasma estradiol, and heat stable phosphatase. The most acceptable measure, however, has been that of estriol determination which appears to embrace both fetal and placental function. Clearly, a simple test which accurately reflects the condition of the placenta and fetus is highly desired. It would allow suitable obstetrical intervention as well as permit monitoring of the effects caused by the introduction of various treatments such as drugs, bed rest, or amniotomy. Dr. Pentti Siiteri of the University of Texas, Dallas, has devised a system which quantifies the metabolic clearance rate (MCR) of dehydroisoandrosterone sulfate (DS). In normal pregnancy, the clearance of DS from the blood rises continuously and it appears that the observed increase is due to the capability of the placenta to extract and metabolize DS. It should be noted that 80 percent of the DS is irreversibly metabolized in the placenta and the MCR of DS (MCR_{DS}) reflects placental clearance due to the following:

(a) placental extraction of maternal plasma DS
(b) aromatization of DS to estrogen
(c) placental blood flow (maternal)

Therefore, this easily measured parameter offers a unique experimental tool to study kinetics of placental clearance. Some suggestive evidence of an abnormal MCR_{DS} was found in association with fetal demise. In a study of 19 pregnant patients with hypertensive disease, 16 patients with progressively rising MCR_{DS} delivered living infants, but in the three women who demonstrated falling values, the fetuses died.

In an effort to shed light on the mechanisms involved in the initiation of labor Dr. Maarten Drost of the University of California used as an experimental model, cattle which exhibit a genetically conditioned syndrome of prolonged gestation. Holstein and Guernsey cows can present a condition in which there is complete failure of parturition to occur. The evidence indicates that the major contribution to this phenomenon are hormonal secretions of the hypothalamopituitary adrenal axis of the fetus. In Guernsey cows, it was noted there was fetal adenohypophyseal aplasia; in Holstein cows, the adrenal cortices of the fetus were shown to be hypoplastic and non-functional. These data confirmed the investigators previous work where he succeeded in prolonging gestation experimentally in sheep by either performing hypophysectomy or bilateral adrenalectomy of the fetus in utero. This work tends to support the hypothesis that the fetus is indispensable in the initiation of labor and indeed may be autonomous in its ability to cause the onset of parturition. Findings such as these open up new avenues to understanding control of prematurity. Efforts to avert labor in the future may be more oriented to managing fetal endocrinologic status and the intrauterine milieu rather than to altering the stoichiometric characteristics of the mother.

In an effort to identify promising strategies and hypotheses which ultimately will lead to control of untimely termination of gestation in humans, the PBIM section sponsored a workshop in November, 1970. Discussions were directed to developing problem solving methods for analyzing signal systems and sub-systems that may be involved in the timing of the length of gestation. Prerequisites necessary for labor to occur, initiating factors evident in early labor, and physiologic alterations appearing during late parturition were some of the complex interactions that the participants considered in some depth. Areas in need of further investigation were also outlined. These include the role of the non-contracting segment of the uterus; a study of whole pre-term uteri to follow vectorial spread of excitation; biological activities of oxytocin, prostaglandin, lectithin, amniotic fluid, fetal steroids, and progesterone. It is planned that a collaborative program will be developed to investigate and test various hypotheses that were presented as being important to understanding the initiation of labor.

Developmental Biology

The Developmental Biology area is concerned with fundamental research relevant to the understanding of human reproductive biology during the perinatal period. The findings of molecular and cellular biology are integrated at increasing levels of organization through the support of research extending from single-celled animals to the biologically complex mammalian system. Emphasis is placed on investigations with mammals as well as on other systems which offer distinct advantages for the study of development from implantation through the first year of life. Some of the scientific accomplishments through grants supported from this area follow.

In recent years there has been a growing awareness of the multitude of mechanisms which in higher organisms must be responsible for genetically caused development of form and function. The studies of Dr. Gluecksohn-Waelsch at the Albert Einstein College of Medicine analyze such mechanisms related to enzyme activity in the developing and differentiating embryo through mutations occurring in the house mouse. The significance of this model developmental genetic system lies in its potential for the analysis of mutational events in the highly organized and complex mammalian system.

In the mouse system the investigator has found that radiation-induced albino mutations cause death of homozygotes within several hours after birth as a result of hypoglycemia. The cause of the severe decrease in blood sugar levels has been identified as the absence of glucose-6-phosphatase activity. The glucose-6-phosphatase activity does not appear until just before the time of birth. Before this time, fetal blood sugar levels are controlled by the maternal organism. At 15 to 19 days of gestation, for instance, the investigator found that the albino fetuses had the same blood glucose level as their normal litter mates. A considerable proportion of homozygotes displayed morphogenetic abnormalities, e.g., of the thymus and of the kidney. One further property of the radiation-induced mutations was the absence of a dosage effect in heterozygotes. This work is important for it provides valuable insight into genetic expression in the clinical situation.

The development of various enzymes is particularly difficult to assess in vivo because of the complexity of the mammalian organism. For this reason Dr. Nemeth at the University of Pennsylvania has developed a feasible in vitro system to be employed in the study of liver enzymes and their controlling mechanisms. Using UDT-glycuronyltransferase activity as a mark of mature function, Dr. Nemeth is investigating the maturation of the chick embryo liver in culture. He has found that the rate at which the enzyme activity develops in the liver cells is independent of the rate of cell proliferation, the rate of DNA or protein synthesis, or replacement of organ structure by parenchymal cell monolayers or aggregates. This precocious enzyme development appears due to the removal of the liver from an inhibitory embryonic environment. These findings contribute to fundamental understanding of liver function during embryonic development.

The enzyme, alkaline phosphatase, is a component of the outer surface of the microvilli of the intestinal epithelial cells. In both mammals and birds, it achieves its high level of activity during brief critical periods that are incited by adreno-cortical secretion. Studies by Dr. Moog at Washington University offer the opportunity to elucidate in detail the series of steps that constitute this differentiation and also the factors that regulate the occurrence and rates of these steps. In the small intestine of the mouse, Dr. Moog and her associates have found that alkaline phosphatase activity is high in the duodenum and falls to a much lower level in the proximal jejunum. The difference is quantitative and reflects a graded distribution of the isozymes of intestinal phosphatase; the factors involved are being sought. Dr. Moog reports that biliary stasis caused a marked decrease in phosphatase activity in the duodenum. It appears likely that specific components of the bile directly influence the character of the phosphatase and its level of activity. Studies such as this relate to the regulation of functional epithelium in the newborn.

Drs. Rao and Johnson at the University of Colorado Medical Center have shown that for multi-nucleate HeLa cells, formed by fusion of cells from different phases of the cell cycle, DNA synthesis and mitosis are inducible. This striking manipulation of mammalian chromosomes in vitro affords new avenues for exploration of the factors inducing cell proliferation. Fusion of the cells of human origin was obtained by the use of UV-inactivated Sendai virus. In heterophasic multinucleate cells formed by fusion of S and G2 cells, the initiation of mitosis in one or more of the advanced nuclei resulted in the premature chromosome condensation of the lagging nucleus or nuclei. Premature condensation could be readily induced in interphase cells by fusing them with mitotic cells. Induction of chromosome condensation in G1 and G2 nuclei resulted in the appearance of chromosomes with single and double chromatids respectively, whereas the S nuclei yielded unevenly condensed chromatin.

A Workshop in Developmental Biology was held in January, 1971. Five leading developmental biologists addressed themselves to key areas that in their view will continue to contribute to the ability of the obstetrician and pediatrician to ensure the well-being of each newborn infant. At the most fundamental level, the discussants placed emphasis on the regulation of gene

activities in the development of multi-cellular organisms. At a more immediate level, the mapping of the human genome merited precedence. Recent advances in somatic cell hybridization make this approach realistic, particularly for the genes that code for developmental proteins. Crucial to further insight into histogenesis and organogenesis is further molecular information concerning the cell membrane during ontogeny. These and a number of other research areas that are relevant not only to the PBIM Branch but also to other Branches in the NICHD Extramural Program were brought forward. Certain areas that are significant in terms of an increased ability to solve developmental problems appear suitable for collaborative mechanisms. Included here are the production and maintenance of mutants that affect fundamental patterns of organization and the formidable but highly rewarding task of mapping the human chromosome.

Fetal Health and Development

The Fetal Health and Development area is responsible for the broad areas of normal fetal growth pertaining to specialization and development of tissues and organs. Emphasis is placed upon such studies as nutritional, immunological, pharmacological, physiological and metabolic events which relate to fetal health and growth from implantation until parturition. Studies on the functional roles of placenta involving feto-maternal gas exchange, feto-placental hemodynamics, drugs and nutritional transfer, fetal-metabolic products, and feto-placental-maternal relationships, are essential in order to understand better the environment and conditions which affect the welfare of the fetus. Likewise, studies of the intrauterine condition and effects of administration of drugs, anesthetics, hormones, or infection, smoking and other maternal malnutrition and abuses on the fetus are of increasing concern. Attention, therefore, must be placed upon the clarification and understanding of the causes, mechanisms, prevention, treatment, etiology and pathophysiology of congenital malformations. Some of the basic approaches by means of chromosomal, biochemical, and biophysical investigations are considered vital not only as bases for fetal status evaluation but also for meaningful antenatal diagnosis.

Some of the selective research accomplishments for the past fiscal year are outlined as follows.

The appearance of a visible morphological defect, which is often used as evidence of the influence of teratogenesis, rarely provides information about its etiology. Dr. D. M. Kochlar of the University of Iowa believes that the understanding of mechanisms which underly experimental teratogenesis is of primary importance. He has developed a culture system which will enable him to determine whether known teratogens act directly on the embryo, whether they affect early cellular differentiation of areas that might be involved in subsequent malformations, and whether they influence the synthesis of substances, such as polysaccharide or collagen, that may play a decisive role in normal or abnormal development.

For the purpose of understanding the role of cell death and mitotic arrest in rat teratogenesis, Dr. James Wilson of the Children's Hospital Research Foundation, Cincinnati, Ohio developed a "split litter" technique. By this

technique it is possible to correlate the embryotoxicity, teratogenicity, embryolethality and growth retardation seen on day 20 with the cellular and molecular events occurring within a few hours or days after the administration of a known teratogenic drug. He further reported that cytosine arabinoside and hydroxyurea, both known to inhibit DNA synthesis, contribute to the abnormal development of the embryo, interfere with mitosis and cause cell death in embryo tissue within hours after their administration in different ways and at different rates.

Dr. Josef Warkany and co-workers of the same Institution are making interesting progress on studies of the effect of various teratogens upon the development of the genital ducts in male and female rat fetuses. They have studied teratogenic effects on rats before mating and the progeny of such rats which show congenital malformations of the central nervous system and of the heart. These rats are then used for studies dealing with the pathogenesis of spina bifida, hydrocephalus, Arnold-Chiari malformation and of complicated cardiovascular anomalies.

Studying the distribution and fate of nicotine in the fetus due to maternal smoking habits, Dr. H. David Mosier of the Memorial Hospital of Long Beach, California made extensive observations on the effect of high levels of nicotine in rat fetal plasma lipid, phospholipid, and liver lipid and cholesterol, and correlated them with the high doses of nicotine administered to the mother during gestation. Nicotine effects have been found to be histologically observable in fetal tissues, especially heart and blood vessels, not only during the gestation period but also for as long as 121 days after birth.

From in vivo and in vitro studies of fetal oxygenation, Dr. Andre Hellegers of Georgetown University, Washington, D.C. reported that by diffusion respiration, the fetus is capable of surviving for approximately three quarters of an hour in the total absence of maternal breathing. This observation led him to study the fetal conditions of oxygen transport particularly those associated with sickle-cell anemia. Dr. Hellegers reports that in sickle-cells he has found the 2,3 DPG content to be increased in amounts which are not imcompatible with changes in oxygen dissociation curves observed in sickled whole blood.

Dr. Jack Metcoff at Oklahoma University analyzed the protein DNA ratio of leukocytes of infants with intrauterine malnutrition (IUM), and found that the cells are larger than those in infants who are appropriate for gestational age (AGA), irrespective of birth weight. Moreover, there was a positive correlation between the cell sizes of the AGA infants and those of their mothers, but this condition was not observed in IUM infants. Dr. Metcoff believes that this new finding may be used to diagnose a fetus suffering from intrauterine malnutrition by examining the leukocytes isolated from the peripheral blood of mothers. Plans are currently underway to continue this study in depth.

Infant Survival and Well Being

Research pertaining to infant survival and well being is concerned with basic and clinical studies of the etiology, pathophysiology, therapy, and

follow-up of conditions and syndromes associated with the postnatal period up to one year of age, such as asphyxia, hypoxia, respiratory distress, hypoglycemia, anemia, erythroblastosis fetalis, sudden infant death syndrome, etc. Of equal importance are the areas involving physiological, biochemical, and behavioral aspects of extrauterine adaptation, including respiratory, cardiovascular, thermodynamic, hematologic, and metabolic changes associated with adjustment to the extrauterine environment.

Interests in this area also include studies of the influence of maternal environmental conditions, events, and treatments as they affect neonatal adaptation; the etiology, pathophysiology, and management of specific congenital malformations observed during the newborn period; and the elucidation of optimal nutritional, environmental, and metabolic requirements for both normal and high-risk infants in the transition to an extrauterine existence. Also of concern to this sub-program area are the effects of those events or therapies occurring during the early days of life upon adaptation and subsequent development, particularly as they involve the high-risk newborn and relate to such conditions as hypoglycemia, hyperbilirubinemia, phototherapy, artificial ventilation, and minimal handling.

Several interesting findings resulting from grants supported in this section are highlighted in the following paragraphs.

Dr. Michael Heyman, a Research Career Development Awardee at the University of California, is investigating the possible pharmacological induction of closure of the ductus arteriosus in premature infants. As a result of his experimental studies with sheep, it has been shown that the premature ductus arteriosus is less responsive to oxygen than in the mature fetal lamb. The ductus does however constrict with acetylcholine. Dr. Heyman is now investigating the use of acetylcholine injected at the aortic orifice of the ductus arteriosus in closing the patent ductus in human premature infants in cardiac failure.

Dr. Roderic Phibbs, another Research Career Development Awardee of the University of California, has continued his follow-up evaluation of infants who received intrauterine transfusions during fetal life. He has found the tendency for functional performance to improve with age. This continued improvement was unexpected and is of particular interest.

The sudden infant death syndrome each year claims the lives of approximately 7,500 babies between the ages of two and six months. The Institute is currently supporting by research grants several investigations which are attempting to define physiological and environmental factors associated with the etiology of this mystery killer.

Dr. Maria Valdes-Dapena at St. Christophers Hospital for Children, Philadelphia, has been awarded a research grant which will enable her to examine the hearts of victims of sudden infant death syndrome for evidence of gradual destruction of tissue that makes up the conductive muscle fibers of the heart. Hearts from infants of the same age whose deaths resulted from recognized causes will also be examined for evidence of similar heart tissue degeneration. This study will confirm or refute a recent

report by Dr. Thomas James, (Amer. J. of Cardiology, 22:479-506, 1968) that focal patches of "resorptive degeneration" were found in 16 infants who died suddenly and unexpectedly. In this report Dr. James speculated that lethal arrythmias or conduction disturbances might possibly result from these developmental changes in myocardial tissue and lead irreversibly to crib death.

Dr. C. George Ray at the Children's Orthopedic Hospital in Seattle has been awarded a research grant to study the association of viral infection with the occurrence of sudden unexplained infant death. Current efforts are directed toward continued monitoring of these crib deaths in King County, Washington, with the aim of defining viral serotypes most frequently associated with such deaths, localizing viral antigens in potentially critical organic areas, and initiating prospective studies of type-specific antiviral antibodies, as well as immunoglobulin studies, among populations of high risk newborn infants.

An interdisciplinary conference on Horizons in Perinatal Research--Implications for Clinical Care was held in December 1970. Program content included sociologic and genetic influences on maternal capabilities and the effects of pollution and infection upon the newborn infant's ability to adapt to the extrauterine environment. A publication of the conference papers and discussion is planned.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Cooperative Hospital Study on the Respiratory Distress Syndrome in Infancy
Contractor : Case Western Reserve University
Money Allocated : $43,893

Objectives: To undertake a collaborative study directed at comparing two regimens of early treatment of respiratory distress syndrome which differ only in the concentration of oxygen (80% versus 40%) in the environment beginning at one hour of age until six hours of age.

Significance of Biomedical Research and Program of the Institute: Idiopathic respiratory distress syndrome represents a major health problem throughout the world. In the United States of America, approximately twenty-five percent of infants born prematurely show signs of this disease within the first 24 hours of life. Twenty to forty percent of these babies succumb from it, accounting for about 25,000 infant deaths annually in this country.

Empiricism, rather than controlled clinical trials, have led to a variety of practices in the management of various aspects of the neonatal respiratory distress syndrome without any marked changes in mortality. It is now recognized that pulmonary hypoperfusion is an important factor in the pathogenesis of the respiratory distress syndrome, and that therapy should be directed to correction of pulmonary arterial flow. Methods which have been shown to be of value in normalizing pulmonary vascular perfusion are: the reduction of hydrogen ion concentration, increased oxygenation, and mechanical inflation of the lung. Because oxygen and pH can be more easily controlled, it is important to evaluate their effects before embarking on a study of mechanical ventilation. It is clear, however, from the findings of many investigators that correction of acidemia is imperative. There is controversy, however, with respect to the use of environmental oxygen supplementation because of the dangers of retrolental fibroplasia. To prevent retinal damage, the arterial oxygen concentration must be kept below the level that stimulates vasoconstriction. The arterial blood oxygen is crucial, not the ambient concentration. Thus, there is a need for a valid statistical design to evaluate the effects of oxygen therapy with careful monitoring of arterial oxygen tension to prevent the development of retrolental fibroplasia.

Proposed Course: Details of the protocol have been designed to provide high-grade intensive care from the earliest possible time; no experimental techniques or medications have been introduced. Each subject admitted to the study must weigh 1000 grams or more at birth; be breathing without assistance by the age of ten minutes; by sixty minutes of age more than 50% of his respiratory activity must be accompanied by grunting, whining or a complaining cry or groan on expiration; be free of any major congenital anomalies which could contribute to respiratory distress; be no older than

one hour and fifteen minutes of age. Once a patient is admitted to the study, the assignment to one of the treatment regimens is made without bias, so that the resulting data will show the relative merit of the two forms of early management.

Arterial blood oxygen concentrations are monitored through umbilical catheterization during the five hours following admission to the study. At six hours, the infant's clinical condition and laboratory values are evaluated using the scoring system developed for this study. This scoring system is recorded at 1, 6, 12, 24, 48, and 72 hours. All infants after six hours are treated according to the same regimen.

All data are submitted to the Department of Biometry at Case Western Reserve University for statistical evaluation.

The sample size is now becoming large enough so that inter- and intra-hospital analysis of data is now feasible. It is not, however, possible to comment on preliminary findings in this report. The clinical collaborators do not have access to the data. To date (3/31/71) 132 infants have been admitted to and retained in the study.

NICHD Annual Report
July 1, 1970 through June 30, 1971
Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Standardization of the African Pygmy Goat as a Laboratory Animal PH-43-67-688
Contractor : University of Oregon Medical School
Money Allocated : $17,414

Objectives: The contract with Dr. James Metcalfe, University of Oregon Medical School, for the development of a Pygmy goat colony as a resource for research purposes was negotiated for a fourth year. Development of this resource will make possible studies in which the growth, developmental, and genetic characteristics of these animals will be measured with a view to evaluating the results of artificial insemination, semen preservation, and ova transplantation in this species, as well as various studies related to perinatal biology, fetal growth and development, and neonatal well being. Studies to date have resulted in the accumulation of data pertinent to the reproductive physiology and husbandry of this unique goat strain. During the contract year a more complete analysis and interpretation of data on oxygen consumption was carried out. Some interesting observations were made. For example, in some mammals, such as humans and lambs, peak oxygen consumption per kg occurs on the second to fourth day after delivery. In the Pygmy goat neonate, peak oxygen consumption has been observed on the first day of life. It has been determined that Pygmy goats respond by a shift in the blood dissociation curve to the right (decreased blood oxygen affinity) which increases the efficiency of the blood in delivering oxygen to peripheral tissues. In humans this shift occurs as a result of increased concentration of 2,3-diphosphoglycerate (2,3-dpg) in the red cells. In Pygmy goats, it is speculated that a structurally different hemoglobin is produced since their red cells do not contain significant amounts of 2,3-dpg.

Significance to Biomedical Research and Program of the Institute: The African Pygmy goat is a dwarf goat which reaches maturity at six months and has a long breeding season. Its adult weight of 50 pounds and height of 60 cm. make it easy to handle (even by female laboratory technicians), cheap to feed, and economical to house. It is an herbivore, trains easily, and lives naturally in herds subject to accepted procedures of animal husbandry. It is anticipated that the development of this animal for research purposes may result in a preferred alternative to the sheep for fundamental research relative to problems of transplacental diffusion, blood flow during pregnancy, prenatal life, parturition, and neonatal adaptation. The increasing interest in the physiology of the fetal-maternal complex demands the development of an experimental animal with uniform physical and genetic characteristics. It would appear that the Pygmy goat may well fulfill these requirements.

Proposed Course: The contract for the standardization of the Pygmy goat will be concluded this year with completion of a handbook entitled "The Biology of the Pygmy Goat". A research grant to Dr. Metcalfe for support of investigations into the effects of progesterone on circulation in the female Pygmy goat was approved by the National Advisory Heart-Lung Council and the National Advisory Child Health and Human Development Council at their March 1971 meeting.

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Growth and Development Branch

The Growth and Development Branch supported approximately 30% of the research grants in the NICHD during FY 1971. This percentage is a decrease from about 35% several years ago. The change is accounted for by the increases in support in other programs within NICHD and the disproportionate increase in average grant size vis-a-vis increases in overall budget. During this fiscal year special attention has been focused on nutrition and on early child development, with these areas representing absolute increases in both number of grants and amount of money invested.

During the past year a significant new book entitled "Issues in Human Development" was published. Adapted from material presented at a conference of the same name, this book explores the biomedical and environmental factors which shape the development of children and adults. Two particularly important sections deal with the effect of urbanization on urbanites and the relationship between man's institutions, economic system, laws and individual development. Another publication was on "The Secretory Immunologic System." This highly technical report brings together the observations of scientists concerned with secretory antibodies in many different organs, with emphasis on the relationship between these and the development of resistance to disease. It is possible the key to immunization against the common cold and to influenza will be found in studies such as those reported here. During the past year the Branch collaborated with the Pan American Health Organization in sponsoring a multidisciplinary conference of Latin American and U.S. scientists concerned with the social factors which influence assessment of behavioral changes in field nutrition studies. The proceedings of this conference are being edited for publication in both Spanish and English.

The "Guide to Nutrition Terminology" has been published and distributed. Developed jointly by NICHD and NIAMD, the Guide is designed to provide a system for indexing, storing, and retrieving nutrition information from the wide range of fundamental, biological, medical, and behavioral disciplines concerned with nutrition health and disease.

Six members of the Branch participated actively in the White House Conference on Children; many other members of the Institute staff also contributed to the planning and conduct of this meeting, which is held every ten years.

Mechanisms of Biological Growth

This area is concerned with the factors and processes which control and promote growth. The number of research projects supported continues to number about 80 since growth of this area has been hampered by an inability to fund many good new projects and approved renewals. About one-half of the projects are basic studies related to growth at the cellular and molecular levels. The remainder are physiological and metabolic studies in higher organisms.

The basic studies which are fundamental to progress in understanding the growth process include studies of protein synthesis and its regulation by genetic, hormonal and other factors, the relationship between structure and function of proteins, the development, regulation and characterization of enzyme ststems, and processes regulating cell structure and differentiation. Of new projects initiated during the current year, two will study gene regulation in development and will contribute to an understanding of the genetic control mechanisms which operate during ontogeny. Another will explore the mechanisms whereby glucocorticoid hormones exert their effect on growth and differentiation at the cellular level. A fourth will study the generation and maintenance of important biological patterns (with particular reference to developmental systems) through the development of theoretical and subsequently experimental model systems. These will utilize various known morphogenetic mechanisms, such as cell sorting and migration, and cell differentiation, first in isolation to determine the resulting patterns, then in superimposition to determine the relative importance of each in the generation of known biological patterns.

The physiological and metabolic studies in higher organisms supported by this area, are concerned largely with elucidation of normal growth and development. The majority are in the field of endocrinology and are concerned with the role of the endocrine system in growth, the mechanisms whereby specific hormones exert their effect, endocrine function as a factor of development, neuroendocrine control mechanisms in growth, and endocrine function in growth retarded states. A new project has been initiated which will study the factors involved in the synthesis, storage and release of growth hormone and prolactin. A particularly interesting aspect of this project is that intact pituitary large granules will be isolated and studied. The structure of the granules, hormonal content, mechanism of synthesis and storage, and factors involved in hormone release from the granule will be studied in preparations derived from normal animals and hormonally manipulated animals.

While the emphasis of the program is not upon the study of specific endocrine and metabolic diseases of children, elucidation of these abnormal conditions often contributes to an understanding of normal growth and development. Thus a number of projects are supported which are exploring disorders affecting growth in clinical populations or animal models. Two new projects initiated this year are representative of this category. They will study the pathogenesis of the hereditary chondrodystrophies in children and in animals at biochemical, genetic and ultrastructural levels. In addition to contributing to knowledge of this disease, they will contribute to an understanding of the early differentiative processes in endochondral ossification and of the formation and structure of cartilage.

Developmental Immunology

The area of developmental immunology is directed toward studies that will elucidate defense mechanisms of the human host in combatting infection and disease. Although there is no categorical disease orientation, efforts are focused on studies of basic mechanisms involved in humoral and cellular immunity as well as an understanding of the local secretory immunologic

system and the various non-specific protective mechanisms. Efforts will be directed toward a holistic approach to the development of immunologic competence. All the various defenses employed by the human host will be investigated so that the totality of the mechanisms involved can be better understood, and a picture of immunologic balance or imbalance in health and disease states will emerge. Special emphasis is given to regulatory factors which control time-linked changes as they occur with increasing age and maturation from the fetal period onward.

The program can be further expanded to study of environmental factors influencing the overall ability of the immunologic system to cope with foreign agents; conditions such as malnutrition will be studied for the relationship between nutrition and infection.

In December 1969, the NICHD sponsored the first conference ever held devoted to the Secretory Immunologic System. Only within the last decade have scientists realized that there is a special defense system for producing antibodies which are found in secretions like tears, saliva and sputum. Secretory immunoglobulins are believed to play an important role in allergic reactions, defense against potentially pathogenic organisms, immunologic deficiency diseases, as well as prophylactic immunization. The exact role of these immunoglobulins must be further identified and conflicting data must be critically analyzed so that knowledge of basic mechanisms can be expanded and applied toward new immunoprophylactic and therapeutic techniques. The proceedings of this conference appeared in published form in May 1971.

One group of investigators is studying aberrations in the metabolism of plasma and tissue proteins as they relate to production of disease states in children. Studies of the function of specific proteins and the mechanisms that control their synthesis are being pursued as well as the ontogeny of plasma protein and tissue protein synthesis.

Interesting studies of antigen-antibody interactions and biochemical processes in children with allergic disorders is being carried out by another laboratory, with particular interest in cell mediators and metabolic disturbances. There is also a focus on the influence of growth and development on these immunochemical processes through longitudinal observations from early infancy.

Another laboratory is investigating the pathophysiology of specific lower respiratory infections in children in order to learn more about significant persistent residual effects after a single acute episode.

NICHD also supports a follow-up study of infants diagnosed by laboratory methods as having congenital rubella during 1964-65. Intensive investigation of this group is revealing important morbidity, epidemiologic and laboratory data. This study will continue for a total follow-up of five years when all final findings will be compiled and will document the extent of the problem and the need for preventive measures.

Nutrition

The number of nutrition research grants supported by the Growth and Development Branch has increased moderately during the past year. Emphasis continues to be placed on further definition of the physiological and social implications of malnutrition during pre- and post-natal stages of rapid development. Current findings clearly show that prenatal malnutrition may have severe, perhaps permanent, consequences on infant and child growth and development. One new project will further investigate the possibility that activity of a specific enzyme system may provide an index of cell growth in vital tissues of the rat subjected to intrauterine and/or postnatal malnutrition. If proven, application of this approach to human populations would be invaluable.

Branch staff have implemented a coordinated series of studies bearing on the multi-faceted problem of potential impact of malnutrition on mental and behavioral development. A number of small animal studies are investigating the cellular and functional consequences for the central nervous system of prenatal and postnatal malnutrition. The possibility that the consequences of malnutrition may be additive over several generations, and not reversible in just one generation of rats, is receiving attention. The subhuman primate offers one avenue for correlating these effects more directly with those in man. One investigator is pursuing a multidisciplinary study of the physiological and behavioral effects on young primates of maternal prenatal malnutrition, particularly protein restriction. One of the most troublesome aspects of evaluating the long-term effects of malnutrition in humans arises from the social conditions which frequently accompany malnutrition. A new grant initiated this year is attempting to differentiate the effects of early social deprivation from the effects of protein malnutrition on later social behavior of the rhesus monkey.

Two grant-supported projects are investigating the interrelationships between socioeconomic class, environmental opportunities, malnutrition in mothers and infants, and behavioral outcomes during the first 5-7 years of life. One of these has just gotten underway. The other reports a reduction in brain size in children born to severely malnourished mothers; subsequent behavioral development is retarded but may partially respond to subsequent rehabilitation with a combined nutrition and education program. However, the nutritional rehabilitation must start very early in life. These studies are being done in a country where breast feeding of infants has been almost entirely abandoned. NICHD is supporting a project in another country where attempts to assess the nutritional impact of prolonged breast feeding are underway. For the first time this study has shown a decrease in the quality of mother's milk as a function of maternal malnutrition, with a consequent adverse effect on infant growth. The results in term of psychological development have not yet been measured. The impact of various naturally-occurring variations in nutritional status on physical growth and health of a rural poverty population in the U.S. also are underway. Long-term assessment of intellectual and behavior development is planned in conjunction with a nutrition improvement program. The funding of new grants has also been accompanied by the development of companion research contracts to investigate other heretofore inadequately understood aspects of the interraction of nutrition, environment, and behavior. These are focusing on hunger and school performance, the

ecological factors that contribute to malnutrition in poverty families, and nutrition and immunological response to infectious agents or diseases of childhood.

An NICHD research grant and Research Career Development awardee, Dr. Myron Winick, Department of Pediatrics, Cornell University Medical Center received the 1970 American Academy of Pediatrics Meade Johnson Award for his achievements in defining that severe malnutrition retards cell division in human brains. As noted above, this biological evidence of the impact of malnutrition on the developing nervous system has had, and will continue to have, enormous implications for our understanding of nutrition, brain development and behavior.

Other NICHD-supported nutrition studies utilizing the multidisciplinary approach continue to provide important new information. For example, one investigator has found that obese human subjects undergoing weight reduction experience behavioral changes which are similar to those of the chronically starved non-obese person. This indicates that weight reduction of the obese may disturb a highly complex physiological equilibrium.

Papers presented by Growth and Development Branch staff at the Eighth World Nutrition Congress held in August 1969 in Prague, Czechoslovakia, and at the Conference on Nutrition and Human Development held in May 1969 at East Lansing, Michigan have been published during the year. The Branch provided financial support to a Symposium on Anthropological Aspects of Human Growth, held in Kyoto, Japan in September 1968 and to the publication of the proceedings of this Symposium entitled "Physical Growth and Body Composition." Several grantees and one staff member from the Branch presented papers which are included in the proceedings. "Issues in Human Development," also published during the year, contains papers written by the Branch staff.

Developmental Behavioral Biology

This area within the Growth and Development Branch is concerned with the biological substrate and mechanisms that mediate behavioral processes. Activities in the past have included coordination of activities from a number of laboratories supported under grant and contract mechanisms to achieve certain objectives that would not have been feasible without this inter-laboratory coordination and cooperation. One product of this cooperation has been the production of a film by Time-Life Films entitled "Rock-a-bye Baby" as part of their series "The World We Live In." This new film portrays the social relevance of many basic research programs supported by the NICHD. Two staff scientists at the NICHD served as scientific consultants in the production of the film.

Further progress has been made on specifying the neural mechanisms and brain structures involved in maternal-social deprivation and in accounting for the abnormal social-emotional behaviors resulting from such early deprivations. One NICHD grantee has performed cerebellar decortications on several maternally-socially deprived monkeys and has eliminated much of the autistic-withdrawn, depressive and rocking behaviors of the adult isolation reared monkey. These dramatic alterations of social behavior have persisted

some 8-12 months after surgery. In addition to the cerebellar studies, this group has demonstrated in their neonatal anoxia program that such early insults result in deficits of behavior on a variety of tasks that reflect impairment of higher mental processes. As a product of the collaborative nature of that research program, another investigator provided quantitative analysis of the electrical activity of the brain in a group of anoxic and normal animals and found evidence of frontal lobe asymmetry which was not present in the normal animals. Further, he found evidence of neurohyperexcitability and hyperreactivity in the anoxic animals which was not present in the normal animals. The findings of neurohyperexcitability are consistent with behavioral hyperactivity reported in the anoxic animals. These two sets of findings suggest that the hyperactivity syndrome in children may have hypoxic episodes during the birth process as one of its precursor's. If hyperactive children show evidence of frontal lobe asymmetry, as reflected in the period analytic descriptors of Burch, then a major advance would have been realized in relating early insults of hypoxia to deficits in attention, memory and hyperactive forms of behavior.

Learning and Cognitive Development

Human learning and cognition play a vital role in all aspects of individual human growth and development and in individual interaction with other humans and with the environment. Through basic research efforts in this area the G&D Branch is acquiring new knowledge and a better understanding of the total intellectual processes, knowledge that will be of significant assistance to educators and to individuals seeking to achieve maximum fulfillment.

Research supported for several years by this Branch has uncovered much that was not previously known about the nature of intellectual functioning, and ways to assess it. For example, it has been demonstrated that intellectual competence cannot be accurately represented by a simple IQ score, or measured solely by tests of verbal, mathematical, or so-called nonverbal abilities, important as these are. Learning and cognitive development in children as reflected in the child's performance also embraces such other dimensions of intelligence as originality, flexibility, and creativity. This Branch continues to seek new and better ways to understand and explain these elusive qualities which are so difficult to measure; considerable developmental work is currently underway at major laboratories throughout the country to more reliably define and assess them.

Research supported by the G&D Branch has shown that intellectual growth cannot be completely understood unless the multiple influences that bear on human cognitive development in spheres other than the traditionally educational are taken into account. Factors operating in the peer group, the family, and the community, as well as aspects of the classroom and school that go beyond curriculum content, influence human learning in significant ways. This expanded conception of the nature of human learning and cognition, and the role of learning environments beyond the school setting was adopted as a major theme for discussion and recommendation by Forum #5, "The Future of Learning: Into the Twenty-first Century."

In this reporting period through the personal services contract mechanism, staff has provided support to the Merrill-Palmer Institute of Detroit, Michigan for the publication of the proceedings of a conference on "Research and Teaching of Infant Development." This conference was designed for representatives of several disciplines relevant to infant development and provided an opportunity for identifying research problems, and for exchange between researchers and practitioners interested in infant development.

In FY 1970 the NICHD supported 92 research grants in the learning and cognitive area. Seventy or about 76% of these grants were the responsibility of the G&D Branch. An analysis of these grants conducted by the Program Statistics Branch and the G&D staff shows that 18 of the grants dealt with the period of infancy, 10 with preschoolers, 20 with school age children (6-18 years), 12 with general childhood, 11 adult, 4 with multiple ages and 17 with animals. In the infancy period most of the studies dealt with sensory processes and general perceptual abilities. In the preschool period most studies were in the area of discrimination learning, verbal learning and concept formation and identification. In the later age periods the grants were rather evenly scattered over the entire list of perception, learning and cognitive functions categories used by the Institute covering such topics as conditioning, generalization and transfer, motivational processes and social learning. Staff continues to analyze the grants supported in these important areas.

Human Communication

This area of the Growth and Development Branch focuses on the biological, psychological, and social processes whereby infants and children receive culturally symbolic and nonsymbolic stimulation, store and/or associate this information with past experience, and appropriately alter and transmit selected portions of this information to others. Special attention has continued to be given to research questions associated with the acquisition and development of the uniquely human and socially important communicative functions of speech, language, reading and their essential components.

During this reporting period there has been a growing interest among researchers in how children develop the language capabilities that they must later use as adults in speaking and in reading. Recently reported G&D supported research with very young infants may well open the way to direct investigations of certain innate versus acquired abilities. For example, one grantee has found that one- and four-month old intants are able to discriminate the acoustic cue underlying the adult phonetic distinction between the consonants "b" and "p". Moreover, and of even more importance, these researchers have found there was a tendency in these subjects toward categorical perception, i.e., the manner in which adults perceive these same sounds. This may prove to be the first evidence that perception, in a linguistic mode, may well be part of the biological make-up of the organism and are operative at an unexpectedly early age.

In addition to the grant program, but an equally important part of the Growth and Development Program's basic biological and behavioral research activity concerning the processes of communication, the Branch has supported a

conference series entitled "Communicating by Language." Through these meetings and the resultant publications the Program has attempted to determine existing and potential directions of study and to identify the roles which various disciplines can and do play in expanding that knowledge, both independently and jointly. The fourth conference in this series, which was held in May 1971, examined the relationship between speech and learning to read. The point of departure for the conference was to examine the contrast between the ease with which children acquire speech and the difficulty they have with reading. By comparing the processes that underlie these two forms of linguistic communication, and by studying the relation between them, staff hopes to understand better why so many who speak and listen well should find it so hard to read and write. The primary aim of the conference was to reveal what is now known about this comparison and by framing the important questions to stimulate useful research. The Branch anticipates that the formal papers and an edited version of the discussion will become a major publication in the conference series.

Social and Personality Development

The area of social and personality development is concerned with the ways children develop from immature, dependent organisms to full participating members of their society. In contrast to the rather well defined area of cognitive development, meaningful and well designed research programs in personality and social development are difficult to develop. There have been fewer projects funded in this area in FY 1971 than in FY 1970, in spite of staff efforts to encourage or develop sound research proposals. Funding limitations appear to be causing an adverse effect on proposals in this area. Similarly, no new projects in the area of personality and social development were funded in FY 1971. This is in spite of the fact that child development experts have recently testified to the importance of new knowledge about the development of childrens' motivation, emotion, and overall social competence as these relate to the social problems of the day. A major difficulty appears to arise from inability to describe rigorous experimental designs.

Training for Research

As of November 30, 1970, the Growth and Development Branch supported 47 training grants for a total amount of $2,767,000, 18 fellowships totalling $154,000, and 40 research career program awards totalling $1,035,000. As compared to the training support provided through the Branch as of January 31, 1970, this reflects an increase of $33,000 in training grants, an increase of $91,000 in research career program awards and a decrease of $245,000 in postdoctoral and special fellowships.

Of the 47 training grants supported by the G&D Branch as of November 30, 1970, 27 were classified as biomedical training programs. Of these 27 grants, 18 were in the area of physiology, 3 in biochemistry, 3 in endocrinology, 2 in genetics, and 1 in developmental immunology.

Of the 47 training grants, 20 were classified as behavioral training programs. Of these 20, 10 were in learning and communicative development, 4 in communication, 3 in social growth, 2 in behavioral biology, and 1 in personality development.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 02303
Contract Title: Foster Care: Potential and Self and Public Images
Contractor: Social Psychiatry Research Associates
San Francisco, California
Dorothy Miller, D.S.W.
Money Allocated: $21,313 (FY 1971)

This contract is supporting analyses of previously collected data on the self-image and the public images of foster parents. It attempts to find out the potential which exists in the general population for the recruitment of new foster parents and to validate existing criteria for the selection of foster parents.

A monograph is in preparation and will be completed in draft form by the end of the contract period. The monograph will discuss the following topics:

(1) The extent to which women in the general population show potential for caring for children not their own, including the extent to which those who are high on potential are similar to or different from existing foster parents.

(2) The social, demographic and child training characteristics of foster parents as compared to the random sample of Baltimore women.

(3) Validation of existing criteria for selection of foster parents, particularly in terms of their values, child training practices, and family organization.

(4) White-Negro differences in child training practices, controlled by social class. Although a good deal has been written about child training practices among white families, little published information exists on child training among Negroes.

(5) Recommendations for foster agency policy.

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N22-2241
Contract Title: Methodology for the Study of Nutrition as Related to Mental Development and Brain Function
Contractor: St. Joseph Hospital, Lancaster, Pennsylvania
David B. Coursin, M.D.
Money Allocated: $34,600

The objective of this contract is to develop standardized test procedures and instrumentation for the electrophysiological assessment of sensory capacity and function for the visual, auditory and somatosensory modalities. The nature of this testing protocol will be designed to maximize information about cortical brain function as it relates to complex cognitive processes. Specific objectives include falsicating a portable multi-sensory (visual, auditory, somatosensory) testing module that can be utilized for field nutritional studies. This module will have timing circuits and programs that will allow multiple scheduling of sensory stimulation. Additionally, demonstration of the validity of conversion techniques from paper to magnetic tape of EEG signals would reduce considerably the cost of EEG nutritional field studies by obviating costly electronic system perhaps in the field. Studies and evoluation of electronic components for the multi-sensory testing module are still in progress.

Contract Number: N22 81512
Contract Title: A Study of Malnutrition and Mental Development in Bogota, Colombia
Contractor: Harvard University, Boston, Massachusetts
Fredrick J. Stare, M.D.
Money Allocated: $122,000 (FY 1971 U.S.-Japan Funds)

This project is investigating the effect of protein-calorie malnutrition in early childhood on intellectual and behavioral development of children living in an urban slum in Bogota, Colombia. The design calls for identification of pairs of siblings, both under 4 years old, one of which is malnourished and the other well nourished. Both siblings will be supplemented with a locally acceptable food for a 5-year period; nutritional, anthropometric, and behavioral tests will be done at intervals. Comparisons will be made between siblings at stated ages and between experimental and control populations. In contrast to other studies, this plan minimizes genetic and social differences between experimental and control subjects.

During this contract period, plans for the full pilot study (to last one year) were formulated and reviewed by an outside panel of experts. Reorganization of the staff and demonstration of the cooperation of the subjects has permitted initiation of the miniature study. It is planned to transfer this project to grant support during the next fiscal year.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: NO1 92257
Contract Title: Study of Chemical Composition of the Human Placenta
Contractor: University of Iowa, Iowa City, Iowa
L. J. Filer, Jr., M.D.
Money Allocated: $43,237 (FY 1971)

The objective of this project is to do a comparative study of the composition and cellularity of placentas, coupled with newborn birth weight, from full-term uncomplicated pregnancies in well nourished and poorly nourished populations. Too little is presently known about placental function, and changes in placental tissue due to malnutrition need to be evaluated in terms of the effect on the fetus. The following types of placental studies are being pursued:

(1) Evaluation for water content, fat, protein, and hydroxyproline

(2) Determination of trace element concentrations

(3) Calculations of cellular characteristics

(a) Total numbers of cells

(b) Indices of cell size

(c) Indices of cell mass

(4) Comparisons of placental/newborn characteristics

This contract involves collaboration of four different institutions. These include The University of Iowa, University of Washington, Tulane University and the Institute of Nutrition for Central America and Panama. Methodology and equipment at all sites have been set up to carry out proper processing of placental specimens in a standardized manner. Specimens are now being received from each, and initial analyses have provided information showing that all specimens are being adequately processed by the various groups. Individual analyses have been carried out on all the specimens received, and comparative findings will be available when adequate numbers of specimens are processed, and proper information is available from both the well nourished and the poorly nourished population groups. These analyses will be completed as the data is obtained.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 81412
Contract Title: Mathematical Quantification of Developmental Electrophysiology of Brain Function (EEG)
Contractor: Tulane University, New Orleans, Louisiana
Bernard Saltzberg, M.Sc.
Money Allocated: None

The primary objective of this contract is to determine whether functional stages of brain development can be identified, described, and quantified by means of mathematical analytic techniques. Time series analysis methods are being applied to electrophysiological signals collected from children. Pattern recognition procedures will then be applied to the descriptors derived from the time series analyses. In addition, efforts are being directed toward determining the sensitivity of mathematical procedures in discriminating among special populations, e.g., the impact of an impoverished environment on brain development, identification of children with learning disorders, and identification of human genetic variations in fraternal and identical twins.

Since the discontinuence of funds the computer facility utilized in this research has been rendered only marginally effective since critical peripheral equipment which was maintained on a rental basis could no longer be paid for and the loss of that equipment has seriously compromised the analytic capability of the Tulane Group. Although, the research grant submitted to continue this line of research received a high priority the high disapproval rate of that study section round resulted in a decile score that would not permit funding. The Saltzberg group remains unfunded to complete the analysis of data it has accummulated. Efforts have been made to provide the essential peripheral equipment through the Federal Government Excess Equipment Utilization plan and some success has been experienced toward restoring the Saltzberg facility to operational capability. Several papers have been written and are either published or in press. Significant advances have been made in developing neuromathematical models for the analyses of the electrical activity of the brain. In particular, techniques have been developed to detect spiking activity in deep brain structures from surface scalp recordings in animals. If these techniques can be perfected for use with humans, then a major advance and contribution would have been achieved which would permit rapid screening of impaired brain functioning, after associated with violent-aggressive behaviors, in children and adults. Future progress and success of this highly unusual team will be dependent upon continued funding.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 71451
Contract Title: Developmental Electrophysiology of Brain Function and Behavior
Contractor: University of Utah, Salt Lake City, Utah
Edward C. Beck, Ph.D. and Robert E. Dustman, Ph.D.
Money Allocated: None

The objectives of this contract are to establish basic data on cortical evoked potentials (CEPs) from the visual, auditory, and somatosensory modalities on normal children age 4-16; to conduct studies on the effects of impoverished environments on children's brain function, as measured by CEPs, and to conduct studies on identical twins. Behavioral studies are being initiated to relate to the CEPs.

Although no FY 1970 funds have been allocated to this contract the principal investigators have continued to analyze and report upon the large volume of data collected during the contract project period. Recent fundings by the Beck group have confirmed earlier findings and extended their observations to other sensory systems in studies on the genetic linkages of cortical evoked responses. In 88 twin pairs and a comparable number of matched, non-twin pairs it was demonstrated that visual, auditory and somatosensory evoked responses of monozygotic twins were more similar in both amplitude and latency characteristics than those recorded from the other two groups. These findings give further support for the necessity of accelerating research programs to determine the genetic base of cortical evoked potential phenomena and electrophysiological phenomena, in general.

Additionally, the Beck group has studied changes in evoked responses as a function of age, sex, and brain hemisphere. Visual, auditory and evoked responses were studied in 80 male and 80 female subjects between the ages of 4 and 86 years. Results indicate that significant evoked response amplitude and peak delay changes take place from childhood through senescence and that many of the changes previously seen in the response of older subjects apparently begin to occur in middle age. Sex comparisons suggest that evoked responses of males are larger than those of females in childhood, but that the reverse is true after teenage years. Hemisphere comparisons tend to confirm earlier findings that the night hemisphere visual evoked responses are greater in amplitude than the left hemisphere responses of subjects in the normal and above normal ranges of intelligence. The above findings have particular implications for the early detection of senescence which may have its precursors as early as the middle years of life.

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 80972
Contract Title: Cross-National Research on the Family
Contractor: Case Western Reserve University, Cleveland, Ohio
Marvin B. Sussman, Ph.D.
Money Allocated: $80,000 (FY 1971)

This contract involves the development of theory and methodology in the relationship between: social structure (modernization), family structure, socialization and the outcomes of the socialization process. The aim is to find out how various types of families function at different levels of modernization so that individual family members gain access to educational, health care, occupational and other institutions and thus achieve overall social competence.

Arrangements have been made with collaborators in some seven countries who are cooperating with the project staff in the testing of relevant theory. Although the project has provided funds, these collaborators have also provided some of their funds for the research. It is expected that this comparison of data from the seven countries will lead to a firmer theoretical base of knowledge about the family.

In the past year this core group of investigators met in Torremolinos, Spain, to make final plans for management and analysis of this complicated body of cross-national data. In the next 14 months analysis is planned which will throw light on how the families function in a variety of developing and developed societies. This will advance knowledge as to what family structures are functional for the optimal development of component family members.

Contract Number: N01 71449
Contract Title: Guide to Nutrition Terminology
Contractor: Vanderbilt University, Nashville, Tennessee
Elizabeth Neige Todhunter, Ph.D.
Money Allocated: None

This contract has been jointly sponsored by NIAMD and NICHD. During the current contract year the "Guide to Nutrition Terminology" has been published and distributed to the scientific community and others concerned with storage of nutrition information.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: NIH-71-2088
Contract Title: Non-Human Primate Breeding Colony
The Regents of the University of California, Davis
Robert E. Stowell, M.D., Ph.D.
Money Allocated: $126,000 (FY 1970)

The purpose of this contract is to create a resource of subhuman primates reared in a standardized environment and of known medical, reproductive and genealogical history, to provide research animals of known quality to investigators whose research is supported by the National Institute of Child Health and Human Development.

Colony development will be supported by the staff and facilities of the contractor's Primate Service Facility. As the colony is developed, biological and behavioral data profiles will be developed for the colony and made a part of each animal's computerized record. Such information will be made available to recipient investigators upon request. Animals of many stages of development including dated pregnancies, embryonic and fetal material, neonates of known gestation, mother-infant pairs, juveniles of known age, as well as certain biopsy material, tissues and fluids, will be made available to selected investigators. Application for animals will be competitive and allocation will be determined by an ad hoc advisory committee who will also advise on colony and contract management. Animals will be shipped to recipient scientists throughout the country, and in addition a limited number of investigators will be accommodated in visiting scientist facilities on-site. Funds from the sale of animals will be used to offset the costs of future contract years.

In the first year, a base breeding colony of 250 rhesus monkeys is being established. Twenty-five percent are being time-mated in indoor specially-monitored facilities. The remainder are housed in out-door group housing and are being random-mated. Of the offspring, 50 percent are being made available to scientists, and 50 per cent are being returned to the colony to provide for future expansion. The data profile system is being initiated and will be developed through consultation and through feedback from recipient investigators. Experience is being gained with the allocation and distribution system, and as investigators's current needs become known, adjustments will be made in kinds of animals developed in order to meet these needs.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number:	N01 50640
Contract Title:	Study of Relationship Between Nutrition and Behavioral Development
Contractor:	Pan American Health Organization, Institute for Nutrition in Central America and Panama Robert Klein, Ph.D.
Money Allocated:	$429,998 (FY 1971)

This contract has been designed to contribute additional knowledge of the relationship between nutritional status and physical, psychological, and social growth. Two pairs of small villages (aldeas) of approximately 650 inhabitants each have been matched on a variety of sociocultural variables. In one village of each pair a dietary supplementation program is provided with special effort made to include in this supplementation program every preschool child and pregnant woman in the village. Incaparina, a high protein food supplement, is made available to every person in the village who wants it. In the control villages medical service is offered, but no food supplementation is provided. Thus a sample of well-nourished preschool age children can be compared on relevant dimensions with a sample of medically treated preschool children raised under the dietary conditions characteristic of the area. Children will be followed from birth until about six years of age, at which time effects of differences in nutrition should be clear.

The long-term study is now well underway in all villages. Cooperation has been excellent. Preliminary data suggest a clearly demonstrable postive effect of the supplement on nutrition status and physical growth of the children. No effects of supplementation on psychological tests have yet been found, but the study has been underway too short a period to expect data of this type. However, pilot studies with children who have recuperated from severe protein-calorie malnutrition have shown that attention span and motivation to perform on tests were less in previously malnourished children than in controls. Studies with infants in the experimental villages similarly suggest that malnutrition correlates with attention span. A full review of all data gathered during the first year of the study will be conducted in December 1971.

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July 1, 1970 through June 30, 1971
Adult Development and Aging Branch

The success of modern science and technology has profoundly changed the medical problems faced by the United States. Spectacular progress has been made in the control of infectious and nutritional diseases though problems still remain particularly among the poor. Because of this progress fewer and fewer young persons are dying. Thus the expectancy of life at birth has increased. In 1900 that expectancy was 49 years. Now it is 70 years. Naturally there are more older persons. In 1900 four percent of the population of this country was over 65. Now ten percent exceed that age. The absolute figures emphasize the change. In 1900 three million persons were over 65. Now 20 million exceed that age.

Medical science continues to advance. The nation now looks forward confidently to the eventual control of cancer. Much is understood about atherosclerosis, and it seems improbable that it will not eventually be controlled. Hypertension is already coming under control. It seems inevitable that the life expectancy at birth will increase.

Two factors have tended to keep the fraction of the population over 65 relatively low in the past. One has been a high mortality rate. The other has been an expanding population containing many young persons. Both these factors will probably change so as to increase the fraction of older persons. The probability for improved mortality was noted above. We must also expect the population explosion to be brought under control.

Even now our mortality rates are such that in a zero growth population we would eventually have 15 percent of the population over 65. Decreased mortality rates are to be expected and would produce an even greater percentage of older persons. Current projections by the Census Bureau are that there will be 40 million persons over 65 years old 50 years from now, and that they may be 12 percent of the population. And that projection is based on the assumption that there will be little improvement in mortality rates.

At present the problems of the elderly are major ones. They affect not only older persons but also society as a whole. Clearly they will become progressively more important.

If atherosclerosis and cancer, now the major causes of mortality in this country, are brought largely under control what will be the health status of the elderly spared those diseases at least until much later in life? The answer to this question lies in the way control over disease is achieved. The causes of disease can be placed in three categories. One category then contains environmental factors--nutrition, infection, etc. Another category contains a host of genetic defects. The third category contains intrinsic aging processes. The nation is gaining control over its health problems by modification of environmental factors. It is beginning to see hope for the control of genetic factors. However, little progress has been made in the understanding of aging processes and even less in the development of methods for mitigating those processes. Thus it appears that the nation is moving toward a population containing a

large fraction of elderly persons disabled by intrinsic aging processes.

The emergence of a population with a very large fraction of persons over 65 creates many problems. Research should be undertaken now so that the nation can meet the current problems and be prepared for the future ones. Congress has charged NICHD with the responsibility for the development and support of research on the medical and related biological, psychological and societal aspects of aging as they affect individuals and society.

The NICHD expenditures in 1971 to carry out this responsibility were.

Mechanism	Number	Funds
Research Grants	60	$3,592,000
Training Grants	25	2,008,000
Fellowships	6	75,000
Research Career Development Awards	7	188,000
Contracts	5	238,670
	103	$6,101,670

This money was spent in response to the Congressional mandate stated above. Obviously the funds expended could not support investigation in much depth in many of the areas falling within that mandate and not being vigorously supported by other funding sources. However, a number of meritorious areas were probed and are discussed here under topical headings.

Diseases and Aging

The study of aging processes is important because they are major determinants of health in the middle years of life and later. They determine health by causing deteriorations in many structures and functions and by contributing to the development of diseases. If medical science is to provide therapeutic measures to deal effectively with many of the diseases of middle and late life, it must develop information on many types of aging processes. Numerous examples of how aging processes contribute to the development of diseases could be given. It appears worthwhile to mention some of them here. Just how they may make their contributions will be described for some diseases later in this report. In some of the examples listed the intrinsic aging component is so large that it is viewed by some as the sole causative process. This is probably unfortunate since it could slow down search for environmental factors in such entities--presbyopia for example.

Aging processes are important in the origin of coronary, cerebral, and other atherosclerosis, many types of cancer, some types of diabetes mellitus, osteoporosis, osteoarthritis, presbyopia, cataracts, glaucoma precipitated by lenticular enlargement, vitreal degeneration precipitating retinal detachment, macular degeneration of retina, presbycusis, senile dementia, emphysema, ovarian failure resulting in the menopause, and impaired resistence to infectious diseases. All of the biologic studies reported in the rest of this report bear on the deteriorations of function and thus the development of disease with age.

Metabolic Processes and Aging

One way of viewing man is to see him as a complex of chemical structures carrying out biochemical transformations mediated in most cases by enzymatic processes. It appears probable that many of the thousands of processes involved become less effective with age. At any rate most of the metabolic processes that have been carefully investigated in the whole man as a function of age decrease in efficacy with time. The deterioration may be evident in the resting levels of appropriate chemical compounds but becomes even more evident when the system concerned is stressed.

For example, there is a tendency for the fasting serum glucose concentration to rise slightly with age if only because the incidence of clinically overt diabetes mellitus increases with age. However, if the body system that processes carbohydrates is stressed, the deterioration in that system is seen to be striking. If the standards for glucose tolerance tests developed for young adults are applied to persons over 50 years of age, then one half of those persons are found to have the type of response that is found in diabetes mellitus. Investigators now have some information on the origin of this disability. Whatever other defects may also be present, it is clear that the amount of insulin released for a given level of serum glucose is less in the old than in the young adult.

The ability of the body to hold serum lipoprotein concentrations at a low level also decreases with age. This is reflected by the rise of serum cholesterol an important component of lipoproteins and particularly of beta lipoproteins. It should be noted that this metabolic defect is evident even in the absence of the application of acute stress to the system involved.

All the deleterious effects associated with the decline with age in the efficacy of carbohydrate and lipoprotein metabolism are not known. Most significant perhaps is the correlation of both these defects with the development of atherosclerosis--the cause of myocardial infarction and many strokes and the major lethal disease in our country.

This discussion has selected two areas of metabolic defect occurring in age. The two chosen are the best known. However, there is evidence of some other such defects occurring with age. It appears probable that many additional similar defects will become known when the appropriate studies are made.

NICHD supports a small amount of research on metabolic impairment with age. It conducted a conference in 1971 dealing with metabolic change with age.

Endocrine Function and Aging

Some types of endocrinologic function are known to decrease with age. The reduction in the pancreatic release of insulin was noted above. Probably many of the various types of endocrine functions will be found to decrease somewhat when the necessary studies are made.

At present the most spectacular loss of endocrine function with age is ovarian failure which produces the menopause. Little work has been done on the

cause of this and little well-controlled work on the desirability and methodology of treatment. The Institute supported a conference on the menopause--mechanisms and treatment--in 1971.

Immunologic Function and Aging

There is a progressive decrease in immunologic competence with age. Currently this appears to be important with regard to both infectious disease and malignant disease.

The poor resistance of the elderly to infection is well known and probably due in part to a reduction in the efficacy of the system controlling circulating antibodies.

Less well known is the decrease in cellular immunity with age. This can be shown experimentally by the decrease in the ability of the old animal to reject transplants of tissue from antigenically different animals. It turns out that this decrease in cellular immunity may be important in the great increase in the occurrence of cancer in the elderly. The immune system becomes unable to destroy the initially small groups of malignant cells that probably come into existence over much of the lifespan and thus permits them to grow into clinically important cancers.

NICHD supports some work on the decay in immunologic competence with age.

Cellular Aspects of Aging

The organism in its developed state can be analyzed in terms of its component cells and extracellular materials and the interactions between those components. Although the adult human contains some sixty trillion cells, there are perhaps only a hundred or so cell types. Furthermore, it is possible to view all cells as falling into two broad classes: cells that never divide after differentiation and cells that frequently divide or that may divide if a need arises.

In the past the cells that did not divide were thought to be the only ones that deteriorated with increasing age. Cell lines that maintained their ability to divide well into adult life were thought to undergo no deterioration with age. Evidence is now accumulating that in the mammal at least normal cell lines with the ability to divide in adult life do deteriorate with age.

There are interesting questions about these lines of dividing cells. Do they deteriorate with age? Are their lifespans limited only by that of the organism in which they live? Or is there some intrinsic process in the cell lines that will eventually lead to their death? Answers to these questions can be sought experimentally both by *in vivo* studies and by *in vitro* studies.

It should be emphasized that there are certain mammalian cell lines that appear to be potentially immortal. They are the cell lines of certain neoplastic cells that have been studied experimentally. These cell lines can be transmitted from animal to animal and maintained alive for much longer

than the lifespan of the species of animal they come from and are maintained in, and, as far as we know, as long as investigators choose to.

Can the same be done with any type of normal, mammalian cell type? Studies to date have disclosed no normal mammalian cell type for which this is possible. Skin, ovary, and bone marrow cells can be serially transplanted from young animal to young animal, but the transplanted cell lines eventually die out. The Institute is currently supporting work on this problem. In one particular interesting study the investigator transplants cells from the mammary glands of young mice into mammary gland fat pads of other young mice whose mammary glands have been removed. He finds that on the first transfer the cells will divide repeatedly to produce a gland of normal size but that the cell line dies out on repeated transfer to new hosts.

Thus the evidence available from *in vivo* studies indicates that normal mammalian cell lines that have the ability to divide in the adult do deteriorate with age and eventually die out. It is apparent that more cell types need to be studied and that the mechanisms involved should be investigated.

The problem of the survival of mammalian cell lines *in vitro* is also being attacked. In the early days of tissue culture it was thought that some cell lines were immortal in such culture. Today there is great doubt that this is true. Some neoplastic cell lines can be propogated indefinitely in tissue culture. Some cell lines that have acquired chromosomal abnormalities in tissue culture can be cultured indefinitely. However, at the present time there is no mammalian cell line that is recognized by all competent investigators as having morphologically normal chromosomes and being able to survive more than a limited period of time in tissue culture.

This became apparent when methodology had progressed to a state where it was possible to maintain chromosomally normal cells for long periods of time in tissue culture. It was then recognized that lines of normal fibroblasts die out after some months of cultivation. The hypothesis that there is something intrinsic in the human fibroblast lines that lead to their deterioration and eventual death was made explicit in 1961. The fact that normal human fibroblasts do not live much longer than eight months in tissue culture has now been confirmed by many scientists.

The Institute is supporting the investigation of the mechanisms underlying the deterioration of fibroblasts in tissue culture over time. It supports the production and distribution of a line of human fibroblasts (WI-38) to interested investigators. These cells are grown to various stages in their *in vitro* lifespan and then stored frozen at sub-zero temperatures. The central supply of such well-characterized cells facilitates work in this area by reducing the labor of individual investigators and by assuring that different investigators can work on the identical cell line when that is appropriate.

The Institute supports by contract a summer course at the W. Alton Jones Cell Science Center, an operating unit of the Tissue Culture Association, Inc. This course is designed to train young investigators interested in techniques of tissue culture important in the study of cellular aging.

The Institute supports several grants dealing with the deterioration of human fibroblasts over time in tissue culture.

Studies like those discussed above may greatly clarify our understanding of many disease processes. For example, they may contribute to our knowledge of some of the degenerative conditions of the eye occurring in middle and old age. Fibroblasts make hyaluronic acid a major constituent of the jelly-like material that fills the eye. This material degenerates with old age impairing vision and sometimes leading to retinal detachment and blindness. Another example is related to the genesis of cancer. WI-38 fibroblasts grown in tissue culture can be transformed much more quickly by SV-40 virus after they have undergone many divisions outside of the body. This transformation is a cellular process similar if not identical to what occurs in the development of malignancy. These data suggest that age changes in old cell lines make them more susceptible to malignant change.

Studies of the changes with time in cells that do not divide after differentiation--the post-mitotic cells--are under way. The best developed research is that on lysosomes and aging pigment.

Lysosomes, intracellular vacuoles containing a wide variety of enzymes, play an important role in the life of cells. They are the cellular organelles responsible for the digestion of phagocytosed particles. Each particle as it is phagocytosed becomes enclosed in a membrane derived from the cell membrane in such a way as to form what is called a phagocytic vacuole. Phagocytic vacuoles fuse with lysosomes to form vacuoles called digestive vacuoles. Within such vacuoles the digestion of phagocytosed particles occurs. Since not all chemical compounds can be digested, residues are left in the vacuoles following digestion. Lipids are particularly prone to accumulate because of the absence of lipolytic enzymes in lysosomes. Some sorts of cells can discharge the vacuoles containing residual material through their cell walls. Other types of cells, however, cannot and vacuoles containing residual material accumulate in them with time. It is now thought that much if not all of the pigment that accumulates in cells with age consists of residual debris left by the action of lysosomes.

There is also evidence that lysosomes may create vacuoles containing the cell's own protoplasm. For example, other organelles such as mitochondria may be seen within vacuoles. In this type of process the lysosome is probably digesting some of the cells own protoplasm. Presumably, the digested protoplasm is replaced by synthetic processes elsewhere in the cell, but a gradual accumulation of residual pigment should result from this process.

The biological significance of this accumulation of pigment is not known. The pigment can come to occupy 10 percent of a cell's volume. It is particularly prominent in the cells of skeletal muscle, heart muscle, and brain and may contribute to the decreased efficacy of those parts with age.

NICHD is supporting research on lysosomal function with age particularly as it bears on the digestion of the cell's own protoplasm.

Aging and Mental Function

The changes that occur in mental function with age are complex. Appropriate tests show impairment beginning as early as the middle years. So common is this deterioration that an adjustment for it is built into the commonly used intelligence test. An individual does not have to score as high on the test to achieve an I.Q. score of say 100 at the age of 70 as he does at the age of 20. Two factors prevent this fact from meaning that the intelligence of many older persons is not powerful. One is that there is great individual variation in intelligence between individuals. A given older person may be much more intelligent than some other given young adult simply because that older person was more intelligent to begin with. In addition, tests do not measure very well judgmental ability that may increase with experience.

Performances on the various components of intelligence tests change differently with age. Verbal performance tends to remain constant over much of the lifespan. Non-verbal performance tends to decrease from a relatively early age in adult life. Not much is known about this. Investigators are beginning to suspect that the verbal tests measure abilities learned long ago and remembered while non-verbal tests measure current ability.

At any rate mental abilities may deteriorate progressively more rapidly late in life. Senile dementias cause incapacitating loss of mental function in about half of the one million old persons institutionalized in the United States. Some of these dementias are due to cerebral atherosclerosis. Others appear to be due to changes in the brain independent of atherosclerosis. Whether these latter dementias result largely from an extension of the processes causing functional decrements earlier in life or are instead due primarily to a different set of factors is not clear. NICHD supports research on the progressive changes in brain function in the adult years of life. This requires studies not only of the elderly but also of young and middle-aged adults since there is a continuum of change across the lifespan. The Institute is funding and providing staff assistance in the preparation of a handbook to summarize current knowledge in this field and to highlight promising leads.

Aging and Society

Changes in birth and death rates have greatly altered the age structure of the population of our country and those of the other highly developed nations. Never before have countries had such high proportions of older persons. This change in age structure has had profound effects on the individuals in society and on society as a whole. Some of the effects are obvious. The totality of the implications of changing age structure has not been thought through.

There is a tendency among some to look upon this as new and unnatural. But change of this type is nothing new. Age structure of population has probably been changing since early paleolithic times. Fourteen years of life may have been the expectancy then and a man of 70 years a rarity. Gradually in the millennia of the last 100,000 years the life expectancy has increased. The increase was gradual at first. An early extension probably came with the neolithic revolution. At the advent of the Christian era the expectancy in favored cultures may have been 30 years. In late modern times there began a

marked increase in lifespan that added about 20 years in highly developed countries in the first half of the present century. There has been little extension of life expectancy in this country in the last two decades.

However, another factor controlling age structure is coming into play. That is the birth rate. Birth rates have been high enough to progressively expand the human population throughout all its history. This will now be limited either by the voluntary activity of man or by the results of his failure to limit it voluntarily. Because of the decreased birth rate the relative numbers of elderly in the population will increase.

This increase, relative and absolute, in the numbers of the aged, which is seen as a most natural thing when one considers the desire of most persons to be alive and well, raises problems which must be considered and met. They are problems very different from the problems considered earlier in this report. They are largely economic and social.

The aging individual in modern society is adjusting to his change in roles at a time when old age commands no particular status. He has financial problems, health problems, and the problem of what to do with himself. These problems are not only the problems of individuals, they are the problems of society.

Little is being done in this area. NICHD this year supported a conference on the demographic aspects of aging to consider in what directions it should move to help solve these problems.

Training

In 1971 NICHD supported the training of 200 students to conduct research on aging. This training was carried out in 27 training programs in university departments and research institutions. Most of the students worked towards a doctoral degree in behavioral or biological sciences. The remainder were post-doctoral students. The distribution of programs and students was as follows:

Programs-27

Behavioral	11	Mixed	3	Biological	13

Students

	Behavioral	128	Biological	72
Predoctoral	108		34	
Post-Doctoral	20		38	

In 1971 NICHD supported by contract a two week summer course in the biology of aging at Stanford University. This is an annual course held each year at a different university. In 1970 it was held at the University of California at San Diego and in 1972 it will be held at the Jackson Laboratories at Bar Harbor. The faculty consists of biologists from a number of different universities. The students are pre- and post-doctoral students also from many different schools. The course covers many aspects of aging at an organismic

and cellular level. Both the faculty and the content of the course differ somewhat from year to year.

NICHD also supported by contract the course on the utilization of tissue culture techniques in the study of aging mentioned earlier in this report.

Longitudinal Studies

Longitudinal studies are of central importance in the study of certain aspects of human aging--both behavioral and biological. At the same time they are extremely expensive and difficult to conduct properly.

In past years NICHD has held conferences on some of the problems involved in longitudinal studies. These conferences were attended by representatives of various Federal agencies and by university and other scientists conducting such studies. These conferences lead to the recommendation that a group of Federal agencies ask the National Research Council to establish a Committee on Longitudinal Research on Health and on Aging to be run by the NRC but funded by cooperating Federal agencies. An appropriate request has been sent to the NRC by the Institute speaking for the interested agencies. The request appears to be receiving favorable consideration.

The purpose of the Committee would be to:

*Provide leadership for the appropriate development of longitudinal studies.

*Set national goals for longitudinal studies and establish priorities among those goals.

*Facilitate liaison between studies and consider the pooling of information evolving from these studies where useful.

*Facilitate the development of methodology and its standardization where appropriate.

*Provide informational services concerning longitudinal studies.

Animal Resources

A major difficulty faced by scientists wishing to investigate aging is the difficulty in obtaining suitable experimental animals of appropriate ages. Commercial breeders have been reluctant to undertake the provision of such animals because of the possibility of financial loss. The Institute is conducting a continuing, systematic program to make possible the provision of suitable animals to investigators studying aging.

Current efforts are directed toward the creation of rodent colonies. Rodents combine the advantages of being mammalian and, to the extent that that implies,

being similar to man along with certain other advantages. They are small and short-lived, and thus larger numbers can be studied across their lifespans than would be possible with most other mammals. In addition rats and mice have been much more thoroughly studied than most mammalian species making unnecessary much work that would otherwise have to be carried out to make possible valid aging studies. The Institute now has a modest contract with the Charles River Laboratories to create a colony of specific-pathogen-free inbred rats (Fischer 344) and sell them to investigators. Much of the eventual cost of this activity will be borne by the money obtained from the sale of the rats. However, some costs such as those relating to the bacteriological, virological, and pathological characterization of the animals and various innovative aspects of animal husbandry particularly important for aging research will continue to be borne by the contract.

The animals in the colony are now about eleven months old. Laboratory rats usually are seriously ill with chronic respiratory disease by the time they are two years old. Their lungs are involved in a chronic inflammatory process and usually contain abscesses. Many different bacterial pathogens are present. The primary cause of the disease is, however, thought to be a mycoplasma. Most laboratory rats, that are not killed, die from this respiratory disease. However a moderate percentage of old rats die from a cardiac disease characterized by myocardial scarring or from a renal disease characterized by both glomerular and tubular damage. Both of these latter diseases are of unknown etiology. Pathologic studies on several rats from the colony killed at six months of age showed no convincing evidence of pulmonary or renal disease but did show areas of myocardial necrosis. Presumably some animals in the colony will eventually develop myocardial scarring.

A contract was let in 1971 to the Charles River Laboratories to create several colonies of mice to supply investigators studying aging. They will be raised under conditions similar to those used for the rats described above. Colonies of two different inbred strains will be raised. There will be one colony of random bred mice.

Eventually a colony of random bred rats will be established.

Aging in Non-Mammalian Organisms

The mission of NICHD with regard to aging is to understand aging processes in man and to lessen their impact where possible. Because of this NICHD supports studies of mammalian aging since it seems almost certain that if man understood aging processes in any mammal thoroughly, he would be a long way toward a complete understanding of human aging. However, tremendously important insights into human processes have been obtained in the past by the study of organisms only distantly related to man. For example, much modern knowledge full of promise for the treatment of inherited disease stems from studies of bacteria. It therefore is important to consider a number of non-mammalian organisms to see if they have special advantages for the study of aging.

Perhaps the most obvious advantage of such organisms is the shortness of life of some of them. The shortest-lived mammals probably live at least two years under good conditions. The lifespan of some invertebrates is measured in days.

The small size of some invertebrates is also an advantage. Large numbers of these animals can be housed economically in small quarters.

Mosquitos may be taken as an example of an animal that may prove useful in the study of aging. Mosquitos can be raised economically in large numbers in an environment free of bacteria and all other organisms. Their lifespan is less than two months. Since their biochemical systems at a very basic level are similar to those of man, knowledge of the mechanisms involved in their aging may contribute to an understanding of aging in man and permit a lessening of its effects. NICHD is supporting investigators studying aging in colonies of mosquitos maintained free of bacteria. Changes with age in biochemical composition, enzymatic activity, and nucleic acid metabolism are being investigated.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 71-265

Contract Title: American Psychological Association Conference on Aging

Contractor: American Psychological Association (Contract Officers: Dr. Carl Eisdorfer and Mr. John McMillan)

Money Allocated: $3,500

Objectives: To bring together a Task Force consisting of one representative from each of the 31 Divisions of APA to plan a group of positive papers on what psychologists could contribute to the study of aging and the welfare of older Americans. From the papers an APA position statement will be drafted for presentation at the White House Conference on Aging of November 1971. They would also be used to stimulate interest and research in problems of aging in the community of psychologists.

The Task Force meeting was held in New York in November 1970, and papers and further meetings were planned. About $1,300 were spent on the meeting, leaving about $1,700 to apply toward the commissioning of papers and a large meeting. The bulk of funds needed for this have been applied for in research conference grant application HD 5895 (Eisdorfer).

Significance for Aging Research: To stimulate interest in aging, and raise its visibility as an area in the eyes of both psychologists and participants in the White House Conference.

Proposed Course: Further funding to be by research grant application HD 5895.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number:

Contract Title: A Contract for a Course on Cell Culture for the Study of Cellular Aging

Contractor: W. Alton Jones Cell Science Center, Lake Placid, New York
(Contract Officer: Dr. Donald Merchant)

Money Allocated: $30,000

Objectives: This course is a key element in a program to develop an area of research addressing the aging phenomenon of the cultured human cell specifically and cellular aging in general. The course will develop and train participants in the techniques concerned with cell culture and will address the state of the art and philosophies related to cellular aging. Twenty-four participants are selected by committee on a competitive basis for the three week course. A limited number of auditors will be admitted and most of these will be obliged to cover their own tuition and related expenses.

Significance for Aging Research: Knowledge of aging processes at the cellular level is an essential element to a comprehensive research and clinical program addressing age-correlated disease and degenerative processes that afflict people in the course of the normal lifespan. This course will provide the best training available in cell culture technique for qualified investigators who require access to additional model systems to proceed effectively with research programs in cell aging.

Proposed Course: Similar courses will be held annually in the future with curriculum adjustments to meet changes in the state of the art and the needs of the program.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 70-2017

Contract Title: Contract to Breed, Rear and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $11,459

Objectives: 1. Meet current and projected demands for senescent laboratory rats reared on a defined diet in a specific pathogen-free environment.

2. Establish a commercial resource of senescent rats for investigators in aging or investigators contemplating research in aging.

3. Demonstrate commercial feasibility of rearing and maintaining standing colonies of aging laboratory animals.

4. Provide an aged laboratory rat in which microbial, histopathological and other significant biological characteristics have been determined to age 24 months.

Significance for Aging Research: A major constraint influencing the development of aging research has been the almost total absence of an aged animal resource sufficiently characterized to meet the unique needs of aging research. The development of a colony of aging laboratory rats under this contract will significantly enhance the quality and quantity of aging research by providing aged animals which are reared in a defined environment on a standardized diet, free of pathogenic organisms, and characterized with regard to age-specific cause of death.

Currently many investigators in aging cannot acquire aged animals short of rearing the animals themselves, nor are they able to maintain aging rats under the laboratory conditions necessary to allow the animals to survive long enough to observe truly senescent change with age. Also, competent young investigators more often than not cannot support aging colonies of rats until they successfully compete for research support. Without this resource many imaginative young investigators will continue to be excluded from research in aging simply because they are unable to identify an aged animal resource which they would use in the studies they propose in aging.

Proposed Course: Contract is to be continued for a minimum of two years with the contract becoming increasingly self-sustaining beginning with year three.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number:

Contract Title: Contract for Production, Characterization, and Distribution of Fibroblasts

Contractor: Stanford University, Stanford, California
(Contract Officer: Dr. Leonard Hayflick)

Money Allocated: $82,544

Objectives: This facility is designed to encourage the development of high quality research in the field of cell aging by providing access to well characterized cultured human cells as well as access to information and the community of investigators studying the phenomenon of aging in the human diploid cell. Starter cultures may be obtained on a noncompetitive basis whereas more extensive needs for material are subject to merit review and competitive allocation of the resource.

Significance for Aging Research: The phenomenon of aging in culture of the human diploid fibroblast-like cell provides a model to investigate mechanisms of cellular aging in general. More directly success in research under cellular aging phenomenon will provide new access for research on most cancers that are age correlated. Specifically in this regard it has been observed that the greater the age of the normal diploid fibroblast in culture the more readily the cells can be transformed by virus. These cell culture systems appear to be reasonable analogs of normal human cell aging and neoplasia phenomena. Thus this contract in the cell aging program in general focuses on insight in aging processes that would contribute to human health and well being in the course of the normal lifespan.

Proposed Course: This contract is anticipated to be a valuable resource for ADAB program for a minimum of several years in the future.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 70-845

Contract Title: Contract to Conduct a Summer Course on the Biology of Aging

Contractor: The Jackson Laboratory, Bar Harbor, Maine
(Contract Officer: Dr. Earl Green, Director)

Money Allocated: $49,995

Objectives: The course is designed to give an overview of biological aging and then to concentrate on aging at the cellular and molecular level. Twenty-five doctoral students from a number of different universities attend lectures given by outstanding biologists also from a number of different universities. The primary aims of the course are to interest biologists chosen as faculty and young biologists chosen as students in aging, and to provide an opportunity for crystallization of concepts on aging and consideration of scientific strategy and tactics in the advance of knowledge in this area. It will also serve as a model for university graduate or advanced undergraduate courses in the biology of aging, and provide for the preparation and discussion of lectures on topics in the biology of aging.

Significance for Aging Research: If the biology of aging can be established as an explicit topic in the minds of excellent biologists, research in aging should be greatly accelerated. The significance of this course will be made evident primarily by the number of applications for support it engenders among the participants, both students and lecturers.

Proposed Course: Similar courses will be held in the future. In general, each course will be held at a different university.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 71-312

Contract Title: Training Program in Molecular Biology for Gerontologists

Contractor: Department of Biology, University of California, La Jolla
(Contract Officer: Dr. Herbert Stern, Chairman)

Money Allocated: $19,078

Objectives: This contract encompasses two main thrusts. First is attendance of four or five selected young gerontological scientists at the Developmental Biological Techniques Summer Workshop held yearly at UCSD under the direction of the Department of Biology (via an NSF contract).

The second is attendance of these scientists, on an individual basis, in Dr. Stern's laboratory at La Jolla. If possible this will be done contiguous with the Summer Workshop. The scientists will learn specific techniques relevant to their own research, under the auspices of individual investigators.

For optimum effectiveness, this training program should last at least one month, and preferably two or three. Flexibility is of course a key factor. This is planned as an open-ended program effected at the mutual convenience and satisfaction of both the individual researcher and the Department of Biology.

Significance for Aging Research: This contract will aid in the training of young investigators interested in aging.

Proposed Course: This contract should be maintained on a continued yearly basis.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 71-2090

Contract Title: Studies in Aging of the Brain

Contractor: University of Colorado Medical Center, Denver
(Contract Officer: Dr. James Austin)

Money Allocated: $31,648

Objectives: Objectives of this contract are to study and clarify the pathogenesis of senile plaques in the aging brain. The contractor will initially pursue studies in four major areas: 1) to clarify, in post-mortem material, the striking increase in silicon (as $Si\ O_2$) found in the Alzheimer plaque and in its central core. 2) to study silicon levels in fluids and tissues of living patients with Alzheimer's disease. 3) to explore in experimental animals the histopathological consequences of localized and generalized increases in silicon. 4) to further chemically characterize the senile plaque, its core and the tangle.

Significance for Aging Research: This contract will increase knowledge about senile dementia.

Proposed Course: A one-year contract, with no plans to continue support via this mechanism. A research grant is planned to carry on this study to completion and the contract mechanism was viewed as a one-year interim means of support.

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number:

Contract Title: Development of Colonies of Inbred, F_1 Hybrid, and Random Bred Laboratory Mice for Research in Aging

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $10,487

Objectives: Aging studies by their inherent nature require animals in various stages of senescence. The unique requirements of studies in aging for aged animals of defined quality has not been met by commercial breeders or other laboratories that provide defined stocks of animals for other research endeavors. It is important therefore that the production of a basic animal model system be encouraged and supported. In this regard a contract has been negotiated with the Charles River Breeding Laboratories, Wilmington, Massachusetts to develop a colony of laboratory mice of three basic genetic patterns (1) two inbred strains (2) an F_1 hybrid of the inbred strains and (3) a random-bred strain. The contract supports both availability of aging animals to investigators and encourages the development of a self-sustaining supply of defined animals that are commensurate with the needs of aging research.

In recent years it has become increasingly evident that the study of aging requires the development of aged well characterized, genetically defined strains of animals of known environmental status.

Genetically defined animals are essential to aging research because of the significance of genetic control to the aging process. The three basic genetic strains to be developed (1) the C57BL/6, (2) the DBA2 inbred strains, (3) F_1 hybrid of the two inbred strains and the (4) random bred ICR/Swiss Webster. These genetic strains will provide model systems amenable to the exercise of maximum genetic control in aging research by providing model systems in which manipulation of controlled difference is inherently possible, reproducibility of genotypes is readily accomplished and predictability of the model system can be established within acceptably narrow ranges. An evolving sophistication in aging research demands that the background interference of varying environmental factors be minimized or rigidly controlled to standardize environmentally the animal used in aging research and to simultaneously promote the long-term survival of the animal through natural senescence.

Several recent studies have clearly demonstrated that the study of the aging process is significantly affected by the introduction of disease, nutritional variables, temperature and humidity extremes. This contract is aimed at rearing a colony of laboratory mice in which the influence of these factors on the aging process will be minimized.

Significance for Aging Research: The easy availability of healthy, well-characterized mice for aging research will facilitate such research. During the first year of the contract costs for maintenance, colony monitoring and laboratory examination of expired animals are $10,487 in total contract cost. In the subsequent years the support for the colony will be decreased until the colony becomes almost self-sustaining from purchases by investigators in aging research.

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Mental Retardation Branch

The Mental Retardation Branch has primary responsibility for research in the biological, behavioral and social aspects of mental retardation within the context of child health and human development. This responsibility embraces all factors--biological, social and behavioral--that influence subnormal development.

The greatest hope for mastery of the problem of mental retardation lies in its prevention, or when this is not possible, in the promotion of the individual's maximum skills through a wide range of habilitative and restorative procedures. The goals of the Mental Retardation Branch then, are the facilitation of research directed toward the identification, prevention, correction and amelioration of mental retardation.

Research Activities:

As of January 1971, the NICHD was supporting 92 grants, totaling approximately $10.1 million for research directly related to mental retardation. This represents an increase of approximately $1.7 million in research grant support within the fiscal year. This increase is the largest actual yearly increase in the history of the Mental Retardation Branch and is the expected response to several years of basic program planning and development of resources and research personnel. A similar pattern of growth, contrary to the prevailing trend, has been experiences in Institute support for training grants, fellowships, and research career development awards in the field of mental retardation. Here, grant support totaled $1.5 million, an increase of $272 thousand over the previous fiscal year.

Review of regular research grant holdings shows a concentration of research activity in the fields of genetics, metabolism and the embryology of development. Over 40% of the regular research grants fall in these areas and are concerned primarily with the etiology and pathophysiology of mental retardation. Program project grants involving team efforts directed toward solution of major mental retardation problems represent approximately 25% of grant holdings. These large and complex grants are concerned with both biomedical and behavioral science research issues and tend to emphasize research on problems at the interface between disciplines. The remainder of research grant activity is divided relatively equally among areas such as nutrition, endocrinology, neurology, and the developmental behavioral sciences.

A group of NICHD-funded investigators from Philadelphia made a number of interesting observations while studying the biochemical consequences of human chromosomal aneuploidy. A series of experiments were performed on patients with Down's Syndrome. Skin fibroblasts from individuals mosaic for trisomy 21 revealed that fibroblast cells from the trisomy 21 line grow differently than euploid cells from the same individual. Long-term cultures of skin fibroblasts led to dramatic increases in the proportion of trisomic cells after several months of serial subculture. In addition, it was observed that there were significant shifts in the proportion of trisomic cells in peripheral blood when the same patient was studied after an interval of several years. A majority of the patients studied showed a decrease in the proportion of trisomic cells while one patient showed increased percentages of trisomic cells. This information seriously challenges the conclusions currently extant in the literature concerning the correlation of the proportions of trisomic cells in cultured tissues of persons with mosaic mongolism with the phenotype of mongolism. Furthermore, the observation that prolonged skin fibroblast culture enhances the proportion of trisomic cells in mosaic tissues suggests that such cultures should be used when looking for trisomic cells not revealed by leukocyte culture.

At the University of Washington, investigators are engaged in intensive studies of the activity of neurons generating the epileptic process in animal models and in humans. The mechanisms by which the abnormal firing of neurons is propagated throughout the nervous system at intervals can now be identified and the synaptic mechanisms involved are clear. The most common cause of the hyperactivity which results, is neonatal or acquired injury. This often stimulates altered synaptic connections with possible abnormal circuit action behaviorally reflected as mental retardation. Knowledge of these processes is leading to new types of therapy. One form, involving the production of subcortical lesions has been markedly effective in reducing seizures in monkeys. These same animals are also being used to test new anticonvulsant drugs.

The impact of pyschosocial deprivation on the intellectual development of infants and very young children is being actively studied by center investigators at the University of Wisconsin. A group of infants, whose mothers and older siblings are characterized by subnormal levels of intellectual performance, minority group status and socioeconomic deprivation have been enrolled in an enriched program of mental stimulation and socialization. The mothers have also received guidance in child-rearing and training for vocational placement. At the age of three years, these children are significantly ahead of a matched control group in mental, social and personality development. They will continue to be tested to determine whether these gains will be sustained through school age.

An investigator at the University of Colorado has learned more about the relationship between malnutrition and impaired biochemical maturation of cerebral tissue both in man and animals. He has demonstrated a deficiency in vitamin A and folic acid in the children of migrant farm workers who function at subnormal levels of intelligence.

Physicians at the Mental Retardation Center, Children's Memorial Hospital in Boston, followed up 137 full-term neonates who developed convulsions during the first 21 days of life. They were followed up to four years of life. At this time, only 52% of the patients were normal. Twenty-eight percent showed neurological deficits which are usually associated with mental retardation and 20% had died. The scientists reported that neonatal EEG could be an important prognostic aid and that the EEG combined with infusion of metabolites suspected of causing the seizure, improves diagnostic capability.

Scientists at the University of Wisconsin Mental Retardation Research Center found that hyperphenylanemia produced by administering phenylalanine intraperitonealy to seven day old rats caused pronounced disaggregation of polysomes of the cerebral cortex. The smaller units are known to be less active in protein synthesis than the larger. Reduction in protein synthesis may be responsible for abnormal brain development.

Mental Retardation Research Centers:

The introduction of preventive health and social measures and ameliorative intervention and treatment techniques on a broad scale, demands fuller understanding of biological and environmental causes and their interaction. The Mental Retardation Research Centers construction program, authorized under P.L. 88-164 was established with this objective in mind.

In the six years since this program was first initiated under the program direction of the National Institute of Child Health and Human Development, twelve centers have been created. Eleven are located on university campuses while the twelfth is closely affiliated with several universities and is on the site of a state institution. Construction has been completed at all but two of the centers (Wisconsin and North Carolina) and at one part of the three site complex at Kansas.

The research center program, rapidly approaching full operational status, represents the major research thrust of this nation to combat mental retardation. Institute grants to the centers for core support in the amount of $4 million for FY 70 have greatly facilitated program development. These grants have provided for administrative and supportive services, common equipment and supplies. Most important, they have enabled the centers to recruit outstanding scientists to develop new research areas.

The research training activities of the centers are highly varied and embrace basic, clinical and applied approaches in the biomedical, behavioral and social aspects of mental retardation and related aspects of human development. Much of the research, particularly that concerned with intellectual deficit associated with poverty and cultural deprivation, has potential for ready application. Since these deficits are developmental in nature, primary focus is directed to factors influencing pregnancy, neonatal life and early child development. Thus, ongoing studies are further defining the role of malnutrition during critical periods of brain growth and development and the possibilities of controlled intervention. Centers at Wisconsin, Kansas, Peabody and North Carolina have initiated environmental enrichment and early stimulation programs for very young infants and preschoolers with promising results. Innovations in language training, behavior modification and curriculum development and parent - child rearing practices are being applied and evaluated for their impact on deprived children. Undergirding these activities are basic behavioral science research efforts in the areas of cognitive style, social and health precursors to learning disability, perceptual, motor and speech skills.

Another element of the centers program recognizes the importance of early detection and diagnosis to prevention and amelioration of mental retardation. Epidemiological study of a total birth cohort in a community has already been completed by researchers at Einstein Medical College, resulting in a better appreciation of incidence and prevalence and the biological and socioenvironmental variables with which mental retardation is associated.

A fairly reliable screening instrument for detection of abnormalities which can be effectively administered by nonprofessional personnel, is being developed by investigators at Colorado. The ability to identify large numbers of children with subtle deficits early in life will permit intervention during crucial phases of growth. Other efforts in these general areas include profiles and population mobility studies in institutions; relationship of high risk factors to learning disability; diagnosis of minimal brain damage; effect of alternative patterns of care on behavior and development.

Studies of the brain, central to mental retardation research, cover a spectrum ranging from developmental processes regulating the earliest types of neuronal interactions to studies of memory, learning, thinking and language. These investigations are attempting to define the biogenetic factors in the development of the structural and functional properties of the brain in normal and aberrant individuals and to clarify the fundamental cognitive processes relevant to diagnosis and treatment of retarded persons. Studies of this kind involve biochemical, electrophysiological, morphological, neurological, behavioral and other disciplines, often working in collaboration. The MR centers are uniquely staffed to mount such multidisciplinary efforts.

Biological research, basic and clinical, form a major part of center activities. On the clinical side, the application of amniocentesis procedures to women with suspect histories, concomitant with advances in biochemical assays and genetics, is already demonstrating preventive potentials. Newly identified inborn errors of metabolism may also prove remediable through further research, and the mass immunization of children and women against infectious diseases like rubella, illustrates additionally promising dimensions of centers research.

The highly practical goals of the research center program however, cannot be achieved without the substrate of fundamental knowledge of biology at the molecular level. Studies of neural tissues, embryonic development, genetic mechanisms, cell growth and fetal endocrinology are examples of such research by center investigators. While less immediate to the problem of mental retardation, the fundamental knowledge to be derived from these areas of study form the bridges to ultimate prevention.

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July 1, 1970 through June 30, 1971
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NO1 92180
Contract Title: Prevention of Prematurity and Developmental Disorders Through Nutritional Supplementation

Contractor: Columbia University School of Public Health

Money Allocated: $388,607

Objective: The purpose of this contract is to carry out a controlled study on the effects of nutritional supplements to pregnant women in the socioeconomically deprived population of Harlem in New York City. Evaluation will be made of the impact of these supplements during various stages of pregnancy on birth weight, infant development, infant mortality and related disorders. Subjects will be drawn from a clinic population considered at high risk of giving birth to premature infants. This project is presumed to have great social and practical significance for improving physical and intellectual development in racial minority and poverty groups and for the prevention of mental retardation and related handicapping conditions.

Methodology: Experimental and control groups will be selected from a clinic population serving the Harlem area according to certain indices of high risk in producing low birth weight infants such as prepregnancy weight, previous history of prematurity, weight gain during pregnancy and age of mother and low protein intake. Other relevant factors including smoking habits, drug usage and trimester of entry into the project will be considered in the analysis. Infants will be assessed developmentally at birth, 26 and 40 weeks of age.

Results: The initial period of this project - from June 1, 1969 to the present, has been devoted to staff recruitment, development and testing of an acceptable food supplement, obtaining community cooperation, working out procedures for clinic and hospital participation and refinement of research methodology, sampling, and design. These problems have been successfully resolved and the enrollment of women into the study and collection of data is under way. It is anticipated that approximately 30 months will be required to complete the proposed enrollment of 1200 women. Of the registrants

satisfying criteria for inclusion in the project, roughly 75 percent have agreed to participate. Very few deliveries have been made to date and no information is available as yet regarding the effect of nutritional supplementation.

Contract Number: NIH-70-2078
Contract Title : Feasibility Study for a Cytogenetic Registry

Contractor : University of Oregon School of Medicine, Portland

Money Allocated: $15,535

Objective : This is a continuation of an existing contract which was initiated originally on February 18, 1970. The cytogenetic registry will identify data by individual case history of individuals with cytogenetic abnormalities. The registry is designed to include aneuploidy and abnormalities of chromosome structure.

The current contract will complete the computer programming of the core of the system, include data from other laboratories, package the programs and provide them to other laboratories to make sure the programs can be used easily by others, design sub-programs to permit the inclusion of additional phenotypic data, and develop and incorporate "banking" features into the overall system. The "banking" indications will permit laboratories to record and ascertain if frozen sera, erythrocytes, leucocytes, transformed lymphoblasts, or cultured fibroblasts are available from the patient and his relatives, or when cytogenetic and/or biochemical genetic data are on record.

Methodology : The following information will be retrieved and stored on individuals who have had chromosome studies: Identifying data (name, birth date, etc.,) precise chromosome data (chromosome numbers and karyotypes, mosaicism, minor variants, etc.), laboratory in which the studies were done, and mode of ascertainment. Family data will be obtained which will permit determination of empiric recurrance risks for chromosomal abnormalities. Information concerning, 1) the presence of Mendelian genetic diseases which might lead to and correlate with chromosome breakage or other anomaly, and 2) the presence of metabolic genetic disorders such as galactosemia, histidemia, among others will be obtained from the patient or family members for linkage studies purposes. An IBM 1130 computer system is used for this study.

Results : This study is in progress. The registry contains over 4000 cases including 315 consecutive cases ascertained for mental retardation and over 300 cases from a single family segregating for a "marked" chromosome 16. To determine the feasibility of long-distance registration,

50 cases from Los Angeles have been incorporated in the registry. The investigator can restrict utilization of part or all of his data in the registry. Confidentiality of the patient is protected by storage of unit number and name on one magnetic disk and storage of unit number and results on another disk.

Contract Number: NIH-71-2096
Contract Title: Comparative Study of Between Laboratory Variation in Human Chromosome Preparation

Contractor: University of Colorado Medical Center

Money Allocated: $15,586

Objectives: The objectives of this study are: (1) to determine the degree of measurable chromosome variation within a laboratory and between laboratories; (2) to determine the factors contributing to this variation; and (3) to determine if this variation can be corrected and comparable values obtained from different laboratories.

Methodology: The contractor will develop guidelines to establish a standard technique for culturing blood for the collaborating laboratories. Using Hungerford's technique, chromosomal preparations will be made to determine if comparable results can be obtained by the collaborating laboratories. In order to detect minor chromosomal variants, each laboratory will obtain peripheral blood from three individuals previously found to have long Y, secondary constrictions in the short arm of chromosome E-17 and giant satellite in a D chromosome. The contractor will test and compare the independently cultured cells for determinations identical to those derived in its own laboratory. If similar results are not obtained, the contractor will determine the cause of variation. This could include travel to the collaborating laboratories to examine culturing methods and/or provision of specimens from a single source to the independent laboratories.

Results: This project was initiated on February 19, 1971. Preliminary results indicate that interlaboratory variations are not statistically significant. This information provides justification for pooling data and permits comparison of individuals by using standardized cytogenetic techniques.

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